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Program in the History of the Biosciences and Biotechnology

Paul Berg, Ph.D.

A STANFORD PROFESSOR'S CAREER IN BIOCHEMISTRY, SCIENCE POLITICS, AND THE BIOTECHNOLOGY INDUSTRY

With Introductions by
Daniel E. Koshland, Jr., Ph.D.
and
Charles Yanofsky, Ph.D.

Interviews Conducted by
Sally Smith Hughes, Ph.D.
in 1997

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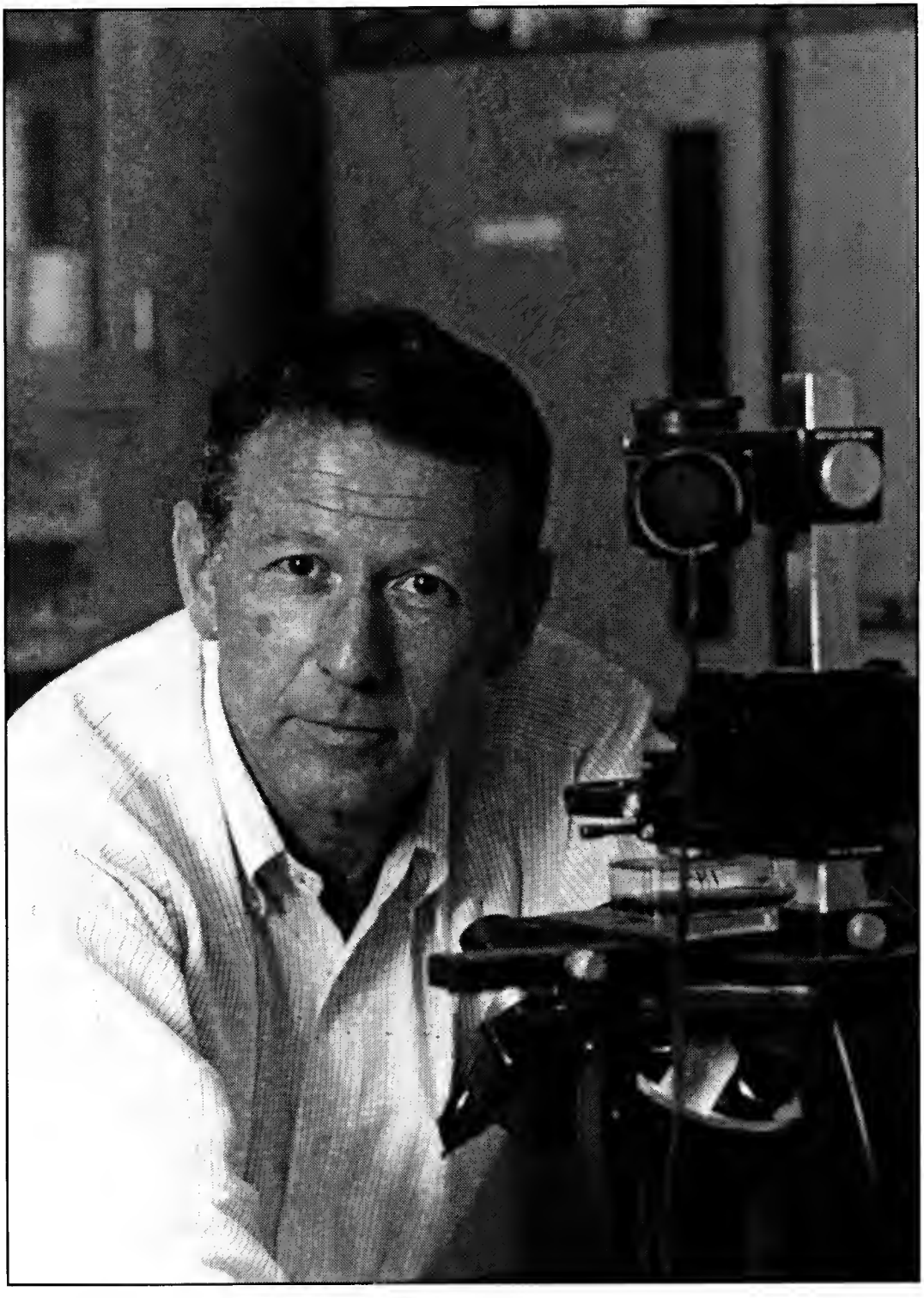

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Introductions by Daniel E. Koshland, Jr., Professor of Molecular and Cell Biology, UC Berkeley, and Charles Yanofsky, Professor of Biological Science, Stanford University.

Interviewed 1997 by Sally Smith Hughes for the Program in the History of the Biological Sciences and Biotechnology, Regional Oral History Office, The Bancroft Library, University of California, Berkeley.

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BIOTECHNOLOGY SERIES HISTORY--Sally Smith Hughes, Ph.D.

Genesis of the Program in the History of the Biological Sciences and Biotechnology

In 1996, a long-held dream of The Bancroft Library came true with the launching of its Program in the History of the Biological Sciences and Biotechnology. For years, Bancroft had wished to document the history of the biological sciences on the Berkeley campus, particularly its contributions to the development of molecular biology. Bancroft has strong holdings in the history of the physical sciences--the papers of E.O. Lawrence, Luis Alvarez, Edwin McMillan, and other campus figures in physics and chemistry, as well as a number of related oral histories. These materials support Berkeley's History of Science faculty, as well as scholars from across the country and around the world.

Although Berkeley is located next to the greatest concentration of biotechnology companies in the world, Bancroft had no coordinated program to document the industry nor its origins in academic biology. For a decade, the staff of the Regional Oral History Office had sought without success to raise funds for an oral history program to record the development of the industry in the San Francisco Bay Area. When Charles Faulhaber arrived in 1995 as Bancroft's new director, he immediately understood the importance of establishing a Bancroft program to capture and preserve the collective memory and papers of university and corporate scientists and the pioneers who created the biotechnology industry. He too saw the importance of documenting the history of a science and industry which influence virtually every field of the life sciences, generate constant public interest and controversy, and raise serious questions of public policy. Preservation of this history was obviously vital for a proper understanding of science and business in the late 20th century.

Bancroft was the ideal location to launch such an historical endeavor. It offered the combination of experienced oral history and archival personnel, and technical resources to execute a coordinated oral history and archival program. It had an established oral history series in the biological sciences, an archival division called the History of Science and Technology Program, and the expertise to develop comprehensive records management plans to safeguard the archives of individuals and businesses making significant contributions to molecular biology and biotechnology. All that was needed was funding.

In April 1996, the dream became reality. Daniel E. Koshland, Jr., provided seed money for a center at the Bancroft Library for historical research on the biological sciences and biotechnology. Thanks to his generous gift, Bancroft has begun to build an integrated collection of research materials--primarily oral history transcripts, personal papers,

and archival collections--related to the history of the biological sciences and biotechnology in university and industry settings. One of the first steps was to create a board composed of distinguished figures in academia and industry who advise on the direction of the oral history and archival components. The Program's initial concentration is on the San Francisco Bay Area and northern California. But its ultimate aim is to document the growth of molecular biology as an independent field of the life sciences, and the subsequent revolution which established biotechnology as a key contribution of American science and industry.

UCSF Library, with its strong holdings in the biomedical sciences, is a collaborator on the archival portion of the Program. David Farrell, Bancroft's new curator of the History of Science and Technology, serves as liaison. UCSF Library contributed the services of Robin Chandler, head of UCSF Archives and Special Collections, who carried out a survey of corporate archives at local biotechnology companies and document collections of Berkeley and UCSF faculty in the biomolecular sciences. The ultimate aim is to ensure that personal papers and business archives are collected, cataloged, and made available for scholarly research, both in paper form and on the Internet.

Project Structure

With the board's advice, Sally Hughes, a science historian at the Regional Oral History Office, began lengthy interviews with Robert Swanson, a co-founder and former CEO of Genentech in South San Francisco; Arthur Kornberg, a Nobel laureate at Stanford; and Paul Berg, also a Stanford Nobel laureate. A short interview was conducted with Niels Reimers of the Stanford and UCSF technology licensing offices. These oral histories and others planned or in progress build upon ones conducted in the early 1990s, under UCSF or Stanford auspices, with scientists at these two universitites.[1] The oral histories offer a factual, contextual, and vivid personal history that enriches the archival collection, adding information that is not usually present in written documents. In turn, the archival collections support and provide depth to the oral history narrations.

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[1] Hughes conducted oral histories with Herbert Boyer, William Rutter, and Keith Yamamoto of UCSF, and with Stanley Cohen of Stanford. The first volume of the oral history with Dr. Rutter is available at the Bancroft and UCSF libraries; transcripts of the other interviews are currently under review by the interviewees.

Primary and Secondary Sources

This oral history program both supports and is supported by the written documentary record. Archival materials provide necessary information for conducting the interviews and also serve as essential resources for researchers using the oral histories. The oral histories orient scholars to key issues and participants. Such orientation is particularly useful to a researcher faced with voluminous, scattered, and unorganized primary sources. This two-way "dialogue" between the documents and the oral histories is essential for valid historical interpretation.

Beginning with the first interviews in 1992, the interviewer has conducted extensive documentary research in both primary and secondary materials. She gratefully acknowledges the generosity of the scientists who have made their personal records available to her: Paul Berg, Stanley Cohen, Arthur Kornberg, William Rutter, Keith Yamamoto. She also thanks the archivists at the Bancroft, UCSF, and Stanford libraries, and personnel at Chiron, Genentech, and Stanford's Office of Technology Licensing, for assistance in using archival collections.

Oral History Process

The oral history methodology used in this program is that of the Regional Oral History office, founded in 1954 and producer of over 1,600 oral histories. The method consists of research in primary and secondary sources; systematic recorded interviews; transcription, light editing by the interviewer, and review and approval by the interviewee; library deposition of bound volumes of transcripts with table of contents, introduction, interview history, and index; cataloging in national on-line library networks (MELVYL, RLIN, and OCLC); and publicity through ROHO news releases and announcements in scientific, medical, and historical journals and newsletters and via the ROHO and UCSF Library web pages.

Oral history as an historical technique has been faulted for its reliance on the vagaries of memory, its distance from the events discussed, and its subjectivity. All three criticisms are valid; hence the necessity for using oral history documents in conjunction with other sources in order to reach a reasonable historical interpretation.[1] Yet these acknowledged weaknesses of oral history, particularly its subjectivity, are also its strength. Often individual perspectives provide information unobtainable through more traditional sources. Oral history in skillful hands provides the context in which events occur--the social, political, economic, and institutional forces which shape the course of events. It also places a personal face on history which

[1] The three criticisms leveled at oral history also apply in many cases to other types of documentary sources.

not only enlivens past events but also helps to explain how individuals affect historical developments.

An advantage of a series of oral histories on a given topic, in this case molecular biology and biotechnology, is that the information each contains is cumulative and interactive. Through individual accounts, a series can present the complexities and interconnections of the larger picture. Thus the whole (the series) is greater than the sum of its parts (the individual oral histories), and should be considered as a totality.

Emerging Themes

Although the oral history program is still in its infancy, several themes are emerging. One is "technology transfer," the complicated process by which scientific discovery moves from the university laboratory to industry where it contributes to the manufacture of commercial products. The oral histories show that this trajectory is seldom a linear process, but rather is influenced by institutional and personal relationships, financial and political climate, and so on.

Another theme is the importance of personality in the conduct of science and industry. These oral histories testify to the fact that who you are, what you have and have not achieved, whom you know, and how you relate have repercussions for the success or failure of an enterprise, whether scientific or commercial. Oral history is probably better than any other methodology for documenting these personal dimensions of history. Its vivid descriptions of personalitites and events not only make history vital and engaging, but also contribute to an understanding of why circumstances occurred in the manner they did.

Molecular biology and biotechnology are fields with high scientific and commercial stakes. As one might expect, the oral histories reveal the complex interweaving of scientific, business, social, and personal factors shaping these fields. The expectation is that the oral histories will serve as fertile ground for research by present and future scholars interested in any number of different aspects of this rich and fascinating history.

Location of the Oral Histories

Copies of the oral histories are available at the Bancroft, UCSF, and UCLA libraries. They also may be purchased at cost through ROHO.

Sally Smith Hughes, Ph.D.
Research Historian

Regional Oral History Office
April 1998

Program in the History of the Biological Sciences and Biotechnology
Completed Oral Histories

September 2000

Paul Berg, Ph.D., *A Stanford Professor's Career in Biochemistry, Science Politics, and the Biotechnology Industry*, 2000

Arthur Kornberg, M.D., *Biochemistry at Stanford, Biotechnology at DNAX*, 1998

Niels Reimers, *Stanford's Office of Technology Licensing and the Cohen/Boyer Cloning Patents*, 1998

William J. Rutter, Ph.D., *The Department of Biochemistry and the Molecular Approach to Biomedicine at the University of California, San Francisco*, 1998

Oral Histories in Process

Horace Barker, Ph.D.
Herbert W. Boyer, Ph.D.
Stanley N. Cohen, M.D.
Daniel E. Koshland, Ph.D.
Marian E. Koshland, Ph.D., retrospective
Reorganization of Biology at UC Berkeley
Edward E. Penhoet, Ph.D.
Robert A. Swanson
Keith R. Yamamoto, Ph.D.

INTRODUCTION--by Daniel E. Koshland, Jr.

Paul Berg is one of the giants of biochemistry in the twentieth century. He made crucial discoveries in the area of intermediary metabolism and then went on to be a leader in the recombinant DNA revolution. Not only was he a major player in the science but also had a pioneering role in resolving the ethical and legal questions that were generated by the new technology.

At a time of increasing controversy, he suggested a moratorium to allow scientists to digest the benefits and hazards of the new vistas of discovery and allow the public to appreciate the balance. That calm moderation in the face of escalating charges and counter charges led to the peaceful resolution of the issue and the guidelines and legislation that have allowed biotechnology to flourish.

This oral history traces Paul Berg's career from his childhood in Brooklyn, through his days as a superstar graduate student (he tries to say it was luck, but as Pasteur pointed out, scientific luck only impacts on history if it connects with a prepared mind), to his days as professor and statesman of biochemistry at Stanford.

The advance of science needs individuals who have the high imagination to solve the complex puzzles of nature and individuals who have the wisdom and humanity to resolve the societal perturbations created by the new discoveries. Occasionally it is fortunate to have all these qualities in one individual. This oral history records the events and thoughts of one such rare individual who was a major actor in the drama of the biological revolution of the twentieth century.

Daniel E. Koshland, Jr.
Professor of Molecular and Cell Biology
University of California, Berkeley

Berkeley, California
July, 2000

INTRODUCTION--by Charles Yanofsky

Paul Berg is an exceptional scientist and individual who is committed to improving everything that concerns him. As his oral history describes, he is responsible for numerous outstanding scientific, educational, and administrative contributions. Throughout his career he identified important unsolved scientific problems, and then proceeded to provide the solutions. But he was not content with concepts alone. Several of his greatest achievements concern technology development and improvement. In addition to his scientific advances, he has made a sincere effort to improve both the support and understanding of science. Few have accomplished as much of significance as he has. For his contributions to science he has received numerous awards, including the Nobel Prize in Chemistry.

I know Paul very well. He and I have been good friends and colleagues at Stanford University for over forty years. I have witnessed many of his scientific, educational, and administrative contributions. We have practiced science and participated in all aspects of academic life in the same university setting. Our families have always been very close and have enjoyed sharing numerous social and other activities. In addition to our interactions with one another, Paul and I joined Alex Zaffaroni and Arthur Kornberg in founding the DNAX Research Institute and in guiding its research and other activities.

If I were searching for a single word to describe Paul based on my knowledge of his activities and achievements, I would choose the word savvy. At scientific seminars and group meetings, Paul listens intently to everything that is presented. He then asks thoughtful and intelligent questions focused on whatever topic is addressed. In group deliberations he is not content until each issue is dealt with fairly, properly, and thoroughly, and at a level reflecting the best thought that can be applied. He states his views and explains his vision clearly and emphatically until the wisdom of his position is evident to all. But he listens to suggestions, and has no difficulty modifying his opinions or incorporating the ideas of others in his recommendations. He has a very positive outlook; I am certain that recipients of his advice appreciate his desire to be helpful.

Paul has played a major role in every enterprise he has become concerned with. At Stanford Medical School he set the highest standard as mentor and practicing researcher. He helped recruit many of Stanford's other "stars," and he introduced several novel courses in our teaching program. He also is largely responsible for the presence on campus of the highly successful Beckman Center for Molecular and Genetic

Medicine. He has directed the center since its founding and has introduced many innovative programs that position researchers at our university at the leading edge in science. He has also played a significant role in educating our country's politicians and administrators on the value of academic research and its potential benefits to society. He enjoyed teaching the subjects he loves, emphasizing their promise for the future, and has written several excellent textbooks that describe our current knowledge in the areas of biochemistry and genetics.

During his career Paul has had to cope with two exceptional more-senior scientists, Harland Wood and Arthur Kornberg, each of whom was always certain that his view was right, regardless of the issue. Paul has been close to Arthur throughout most of his scientific career and has learned how to benefit from the genius of this extraordinary human being. I suspect that there probably is no subject that Paul and Arthur have not discussed or argued about with one another.

The scientists I know well who have worked with Paul have enormous respect and admiration for him. He always had the ability to think beyond their immediate projects and could readily identify the more significant implications of their studies; routine research was never his objective. As you will see in his history, his research interests changed with time, generally reflecting his desire to exploit new and exciting developments in the areas of science that interest him. But he could not ignore potential dangers from the actions of the scientific community, hence his active participation and leadership at the Asilomar conference that recommended restraints on recombinant DNA research.

In appropriate situations Paul can be very competitive since he is always determined to do his very best. This was most evident to me on the tennis court; we played tennis together regularly on weekends for over forty years. We both enjoyed this diversion as an opportunity to get our minds completely off science and to compete on even terms. However, we could not escape our love of science and invariably discussed our respective research programs with one another, while sitting on the bench. It was great fun playing against him, or with him, because of his strong desire to win.

Paul enjoys music, art, the theater, and literature, and collects and displays his preferences in modern art. Paul's wife Millie, a lovely woman, understands his dedication to his many activities and gives him her wholehearted, enthusiastic support. But she also expresses her point of view emphatically, regardless of whether or not she agrees with him. They are fortunate that their son John and his family live close by; they share many interests and activities.

Paul's lifetime experiences undoubtedly influenced his personal goals and achievements. But success came to Paul Berg because he is

very smart, and he is determined. Few individuals have accomplished as much as he has. We are all fortunate that someone with his ability has been so dedicated to increasing our scientific knowledge and improving our quality of life.

Charles Yanofsky
Department of Biological Sciences
Stanford University

Stanford, California
September 18, 2000

INTERVIEW HISTORY--Paul Berg

The subject of this informative and revealing oral history will be familiar to virtually anyone in biochemistry and molecular biology. Even before the Nobel Prize in Chemistry thrust him definitively onto the scientific world stage in 1980, Paul Berg was a prominent figure. He had already established a distinguished record in biochemistry and, more recently, in tumor virus research. He had also risen to international prominence in science politics as a prime scientific spokesman in the controversy of the 1970s over the safety and regulation of recombinant DNA research.

Professor Berg is an old hand at giving an oral history. In the 1970s, he was twice interviewed for MIT's oral history project on the biohazards controversy, a series noteworthy because it encompasses interviews with major figures in the controversy as it unfolded and documents participants' initial ideas about the scientific and commercial possibilities of the new technology. Because of this previous coverage, the present oral history accentuates other aspects of Professor Berg's productive career--his science, his vision as director of Stanford's Beckman Center of Molecular and Genetic Medicine, and his affiliation with DNAX, a private research institute (now owned by the pharmaceutical company Schering-Plough) which he and Stanford colleagues Arthur Kornberg and Charles Yanofsky founded in 1980. The recombinant DNA controversy is nonetheless a presence in this oral history, as Berg reflects on how it influenced the course of research in the field and his nomination for the Nobel Prize.

Inevitably, when one rises to a position of power and prominence in science, or any other walk of life, controversy follows. Berg of course is not immune. There has been debate about the wisdom and significance of the temporary moratorium which scientists themselves, with Berg in the lead, placed on certain kinds of recombinant DNA research in the 1970s and in their formulation of research guidelines. The award of the Nobel Prize, as is so often the case in its century-long history, has also been questioned in terms of those singled out for the honor.[1] Berg describes in detail the contributions of his laboratory to the techniques for joining pieces of DNA by artificial cohesive ends ("A-T tailing") and how this work relates to that of Peter Lobban, Vittorio Sgaramella, and, especially Stanley N. Cohen and Herbert W. Boyer. The reader will likely be fascinated by Berg's comments on these and other controversial topics.

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[1] In additon to Berg, the 1980 Nobel Prize in Chemistry went to Frederick Sanger and Walter Gilbert for the development of DNA sequencing techniques.

There are several surprises in Berg's narrative. One is that he considers his early research in nucleic acid and protein biochemistry to be at least as significant as his later work with tumor viruses used as probes to study the structure, expression, and regulation of mammalian genes.

A second surprise is that several years before the now-famous Asilomar Conference of 1975 on Recombinant DNA Molecules, Berg was sufficiently concerned about the risk of biohazards arising from the growing technical capacity to manipulate DNA that he organized a conference on the topic. Most of the participants in the earlier conference, entitled Biohazards in Biological Research, Berg invited to attend the later conference.

A third surprise, at least to those not intimately familiar with the history of recombinant DNA technology, is that Berg makes repeatedly explicit that he makes no claim to the development of molecular cloning, an achievement which he openly concedes to Stanley Cohen and Herbert Boyer. Although Gobind Khorana and others had previously joined DNA molecules synthetically, Berg claims for his own laboratory the development of technology for using mammalian viruses to carry foreign genes into animal cells. The Berg group used this "gene-splicing" technology from 1972 on to study the dauntingly complex structure and function of mammalian genes. Berg goes on to describe the genesis of the Beckman Center for Molecular and Genetic Medicine and its goal of generating biomedical knowledge which can be translated into clinical application.

Berg's demanding and sometimes contentious career has in no way diminished his energetic style and enthusiasm for a diversity of interests. To this writer, he comes across as friendly, upbeat, and very likeable. He is also self-confident, assertive, and articulate. He is widely known in his public and private lives as a concerned statesman of science and a socially responsible citizen.

The Oral History Process

When I approached Professor Berg in late 1997 about conducting an oral history, he expressed concern about taking time away from his project at Stanford's Center for Advanced Study in the Behavioral Sciences to write a biography, with colleague and friend Maxine Singer, of the biochemist George Beadle. He agreed to an oral history with the proviso that the interviews be limited to four. Although we held to

that number, the duration of each interview expanded as Berg became increasingly engaged in telling his story. My previous research for interviews with Arthur Kornberg and the review I made of the rich collection of Berg's correspondence, both collections archived in Stanford's Green Library, provided institutional context and a basis for questions. In the discussion of his research contributions, Berg the educator and translator of science shines through in the clarity and completeness of his answers, including the diagrams of enzymatic reactions he created as he reviewed the transcripts. His opinion that his greatest contribution is to have taught several generations of young scientists indicates the centrality of teaching in his lengthy list of accomplishments.

Although an oral history is assuredly not the best means for determining the factual content of a scientist's research--published papers, lectures and so on are a far better source--Berg's clear and detailed description of his somewhat arcane research is accessible to readers with only a smattering of biological knowledge. The four interviews were conducted at Stanford between July 15 and November 5, 1997, the first two in Berg's office in the Beckman Center. The last two sessions were taped in his study at the Center for Advanced Study, against a background of the raucous cries of California blue jays in the oaks dotting the bucolic setting above the Stanford campus. The two final interviews were interrupted for lunch on the center's sunny patio, where fellows are expected to gather once a day for scholarly and social exchange. Berg was characteristically disturbed--an indication of his sense of social responsibility--that myriad duties had kept him from the center more often than he had wished.

The lightly edited transcripts were sent to Berg who painstakingly edited and amended them, despite his distaste for the task. In a letter accompanying the returned transcripts (a sample of his editing may be found in the appendix), he remarked: "I thought the ordeal would never end but it has, I'm sure, much to your relief. Sitting those many hours with you was a pleasure which is more than I can say about the many more hours spent reading the transcript: nearly three hundred [double-spaced] pages was almost more than I or anyone should be expected to bear." The fact that he not only bore but considerably improved the content and clarity of his narrative is a gift for which I am, and future scholars will be, highly grateful and appreciative.

In 1998, Berg retired from his professorship but continues to direct the Center for Molecular and Genetic Medicine and to be an active force in American and international science and politics.

The Regional Oral History Office was established in 1954 to augment through tape-recorded memoirs the Library's materials on the history of California and the West. Copies of all interviews are available for research use in The Bancroft Library and in the UCLA

Department of Special Collections. The office is under the direction of Willa K. Baum, Division Head, and the administrative direction of Charles B. Faulhaber, James D. Hart Director of The Bancroft Library, University of California, Berkeley.

Sally Smith Hughes, Ph.D.
Research Historian and Program Director

July 19, 2000
Regional Oral History Office
The Bancroft Library
University of California, Berkeley

Regional Oral History Office
Room 486 The Bancroft Library

University of California
Berkeley, California 94720

BIOGRAPHICAL INFORMATION

(Please write clearly. Use black ink.)

Your full name Paul Berg

Date of birth 6/30/26 Birthplace Brooklyn, New York

Father's full name Harry Berg

Occupation Manufacture of fur clothing Birthplace Suburb of Minsk, Russia

Mother's full name Sarah Brodsky

Occupation None Birthplace Suburb of Minsk, Russia

Your spouse Mildred Levy

Occupation Res Nurse Birthplace Queens N.Y.

Your children John Alexander

Where did you grow up? Brooklyn until 1943

Present community Stanford, Ca

Education BS Penn State Univ (1948), PhD Western Reserve Univ (1952)

Occupation(s) Biochemist Professor at Stanford Med Ctr

Areas of expertise Biochemistry, molecular biology, genetics

Other interests or activities Competitive sports, art collecting,
literature & Theater

Organizations in which you are active Too many to list

INTERVIEW WITH PAUL BERG, PH.D.

I CHILDHOOD AND EDUCATION

[Interview 1: July 15, 1997]##[1]

Family and Early Education

Hughes: Let's start with your birth and upbringing.

Berg: I was born in Brooklyn, New York, on June 30, 1926. My mother, Sarah Brodsky, tells me, a very, very scorching day. The reason I happen to remember is because she raises it each time we have an anniversary. It happened on June 30, she will say, "just like the day you were born."

My parents had immigrated from Russia, and under rather unusual circumstances. I think it was 1919 that they married. My mother was eighteen; my father, Harry Berg, was nineteen, and they left the very next morning, never to see anybody in their families again. They literally worked their way across Europe over a period of three years. They had a child and eventually came to the United States in 1922, I guess it was, arriving in New York and then settling in New York.

Hughes: Was New York their destiny when they started out?

Berg: I think so, because there were some members of the family, a half sister, who was living already in New York City. And there must have been other people who had come earlier from the same little town that they were from, a village outside of Minsk. So they formed a local community that knew each other from Russia, or that knew of each other from Russia.

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[1]## This symbol indicates that a tape or tape segment has begun or ended. A guide to the tapes follows the transcript.

My father, I think, worked for other people for a while, and ultimately started his own little business, which was making fur trimming on coats and collars and hats and things of that sort. The boy that they had while traveling in Europe died not long after they arrived in this country, of what, I don't remember. And so I was born some four years after they arrived.

We lived in Brooklyn and I can't remember a heck of a lot about my early childhood. I was told that I didn't speak English until I went to school, that I spoke mostly Yiddish, which is what my parents spoke to each other. But once I went to school, I very quickly almost lost--well, I didn't lose the ability to understand Yiddish, although I lost the ability to speak it. We lived in what is now the Brownsville section of Brooklyn, a pretty tough neighborhood; at that time it was still reasonably nice.

I went through elementary school, probably through the fourth grade, in that location. I can't remember very much about school. It was certainly not challenging or that I felt strapped. It was easy; I enjoyed it; it was fun. And then we moved to a place in Brooklyn called Sea Gate.

Sea Gate is a small community, which at one time was very exclusive and very private. It's out at the very end of the peninsula which forms Coney Island. There's a little peninsula that comes out from the southern part of Brooklyn, the burrough, and out at the very tip is this little enclosure called Sea Gate. In the 1920's, it was probably quite an exclusive resort, a summer place for wealthy people to come to, because the homes were very large.

By the time we moved there, those homes had become more rooming houses, and it was nothing very special, other than a great place to grow up. During the wintertime it was literally empty. So it was a very small community of young people that I knew and my family knew. In the summertime it was inundated by summer holiday people. But the beaches were right there, so we literally grew up at the seashore. I think we moved there before I reached the fifth grade, because I think I was nine or ten when I moved there. So that was probably somewhere around the fourth grade.

Hughes: Did that move represent a rise in the family fortunes?

Berg: No. I can't really remember exactly. We went there for the summer, again as one of the summer crowd, and I guess my father and mother liked it so much, they decided to move there. We didn't have a house; we rented an apartment, and we lived in that apartment. I then finished elementary school in Coney Island.

There were no schools in Sea Gate, so you had to walk; it was probably half a mile, something of that length.

I graduated from that school and started junior high school. Today, it's called middle school, I guess. It was the seventh, eighth, and ninth grades. I had done very well in school; I had skipped grades twice during this period. I guess that's not common anymore. I forget which ones I skipped, but by the time I was in the sixth grade and went to junior high school, I was already a year ahead. There I entered rapid advance classes, which took the seventh and eighth grade in one year. So you took 7A and 7B in one half year, and 8A and 8B the second half of the year.

That was one of the exhilarating periods of my life. The people who were selected to participate in these rapid advance classes were all very bright and very energetic, and so it was an exceedingly exhilarating period. Because the teachers didn't have to worry about people who were slow in picking up on things, you could do things in that class that you couldn't do in ordinary classes.

Hughes: Do you remember being interested in anything in particular?

Berg: I was interested in science right from the beginning. Even before junior high school, I was interested primarily in biology. I was interested in trying to understand living things. Every time I found any kind of an animal that had died, I took it home to dissect it and see what I could learn about it. I knew that biology was the focus; that was the thing I wanted. Probably at that early stage, it was translated into an ambition to be a doctor.

From the things we read during this junior high school experience, there was a lot of motivation and idealization of being a physician, or a research physician in particular. I ask my students today how many have read Sinclair Lewis's *Arrowsmith* or [Paul] de Kruif's book, *Microbe Hunters*. None of my students have ever heard of either one of them, which is a disappointment. But the key figures that were created or converted into idols were physicians who were doing research and solving major health problems. So I had in mind that I probably wanted to be a doctor, but I didn't necessarily think of it as practicing medicine as much as doing research in medicine.

Hughes: What did your parents say about all this?

Berg: My parents had never had any formal schooling. They left this small town before they had any significant amount of schooling,

other then probably just grade schooling. They could read and write. But there was nothing but encouragement, nothing but strokes, lots of admiration. They knew I was doing well. They never ever queried me about what I wanted to be or anything like that. They just took great pleasure in having their son being successful in something which they regarded as very, very important--education at a very high level--even though they never had it.

I think that was true of many of the immigrant Jewish families. Education was put on a very high level, even though the parents had little of it themselves. My wife, Mildred Levy, says today, because she met my parents when we were married, "You were so fortunate, because your parents not only encouraged you, but they gave you enormous positive feedback. So all the while you were extremely secure in what you were doing, feeling good about it. Without their bragging about it or anything like that, you just knew you were doing well. Your parents thought well of you, and that was great. You didn't have to impress them."

Abraham Lincoln High School

Berg: At the same time, I was very active in sports and very much involved in playing football. Whatever the sport of the season was, I was actively involved. And so when I went to high school, I really wanted to play football as well as do what I had to do in school. But because I had skipped so much, I was really much younger than people who were at the equivalent level in school, so I wasn't as fully physically developed. I think I was undersized and would probably have gotten killed if I went out to play football.

Hughes: You were how old?

Berg: I graduated in January of 1943. So I went to high school three years earlier than that. That's 1940, so I was not quite fourteen. And high school was fun. I didn't take it seriously. I wasn't intensely focused on books. I just found it easy, and I could do lots of other things.

But all that time I was very interested in biology. The biology course was one of the really exciting and interesting things we did in junior high. We did a lot biology. Because of the quality of the students, we did all kinds of projects.

Hughes: What about the quality of the teachers?

Berg: My recollection is that the teacher that we had in junior high school for the rapid advance classes was magnificent. She really knew how to handle gifted kids. She didn't hold us back. She encouraged us. I don't remember so much about her, or him, I'm not even sure which one it was.

The teacher in high school had a very different style of encouraging young people. We were given lots of projects to do, and the projects were to be done at your initiative, and you weren't given a lot of help. There was an enormous amount of interchange amongst the students in the class, and we all knew each other. Many of us were growing up in the same little community. We always regarded ourselves as a special group, because we lived together during the winter when nobody else was there. And we were the "in" crowd, the real crowd.

During the time I was growing up, we had a football team, on which I played. We played in a league, and we played tournaments. My brother, Jack, who is a year and half younger than I am, was also on the football team, and also was a very good student. We were very close.

Hughes: Just the two of you?

Berg: We had a third brother, Irving, who was five years behind me. At that age, I think, we had nothing to do with him. There was just Jack and myself.

When I went to high school, I took all the science courses that were available. But, again, I don't remember knocking myself out; I certainly wasn't an honor student; my name isn't on the board as one of the great students--like Arthur Kornberg's name is there.[1] We went to the same high school. I couldn't play contact sports, because I was not up to that. So I just played the kinds of sports we did at home.

Sophie Wolf

Berg: There was a woman, Sophie Wolf, who was probably one of the more important figures in my life in terms of motivation. She was not a teacher, but she ran the supply room for providing the

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[1] See Arthur Kornberg, *Biochemistry at Stanford and Biotechnology at DNAX*, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 1998.

demonstrations and the microscopes for biology and the models for various lectures. There was this very large stockroom, which had an enormous amount of stored resource material--microscopes, and models of different organisms, and things of that sort.

She had a keen interest in young people. She ran what was called the Biology Club, which met in people's spare time, after classes were over. And so I used to spend the afternoons there at the Biology Club. One of the terrific things about her was, she never gave you any answers, no matter what you asked her. She would come back with a leading question that would perhaps help you go find out on your own. This even included doing experiments, so that if you asked a question which she thought you could answer by actually doing an experiment, she would help you to find out what kind of an experiment you might do. But in the end, it was your drive that led you to do those kinds of experiments. I think during the two or three years that I was in involved in this club, she was constantly stimulating us with questions and leading us on to trying to learn new things.

Interestingly enough, Sophie was also there at the time Kornberg was a high school student. At the time I got the Nobel Prize [1980], somebody asked me if there was any notable person who played a key role in stimulating me, and I mentioned Sophie. I thought she was long since dead, but as it turned out she was retired and living in Florida. The education reporter for the *New York Times* wrote a very long article, because it was unusual for anybody to identify a high school figure as being such a key person. Usually, people identify a university professor who played a key role in moving them along. She then literally came out of anonymity and became quite well known.

Subsequently, a third person from Abraham Lincoln High School got the Nobel Prize, in addition to Kornberg who received the prize in 1959, a man by the name of Jerome Karle, who did some very magnificent work in crystallography. So some time ago, I forget exactly what year it was, they decided to name the science wing of the high school--it was a very large high school--the Sophie Wolf Wing. And one floor is named the Paul Berg floor, and the other one is Arthur Kornberg, and the other is Jerome Karle.

They had this huge assembly; all the students were gathered in this large auditorium, and the mayor and the secretary for education, or whatever it was, for the City of New York came. And there was little, old, wiry, Sophie Wolf, who was pretty far out of it most of the time, but she just lapped up the accolades.

Hughes: There can't be too many individuals at the high school level who are able to boast of mentoring three Nobel prize winners.

Berg: I don't know if it was the only high school in the U.S. that has had three Nobel graduates, maybe the Bronx High School of Science or Clinton High School, which were also very academically oriented. But my school was not specially notable for its academic achievements. It was big. It was located in an area called Brighton Beach, which was quite a way from where I lived. We needed a bicycle, or in the wintertime there was a bus that took us, and sometimes we even walked. It was probably three to four miles.

Hughes: How did Sophie Wolf get her scientific knowledge?

Berg: I think just on the job. I don't know how she got into that role.

The high school opened in 1930. I think Kornberg moved to it from another high school because it was closer to his home. And I think he only went there the final two years of his high school stay. He graduated high school at a very young age. I think he graduated from City College when he was sixteen. So, it's conceivable that he went to high school when he was twelve or thirteen.

But in any case, Arthur acknowledges the impact that Sophie Wolf had. Jerome Karle did as well. She not only was for biology, but she managed the storeroom for all the demonstrations for physics, chemistry, and so on. I don't know whether she had any science background at all. She must have been there right from the beginning when the school opened. But it was a central role, because she saw all the science students. And for anybody who expressed an interest, she would invite them to join one or another of the clubs that she had.

Hughes: It had to be by her invitation?

Berg: Well, either an invitation or encouragement, one or the other.

Anyway, while I was in high school, Pearl Harbor occurred. I graduated in 1943, and I remember very clearly the big assembly. The school was assembled in this large auditorium to listen to President Roosevelt condemn this "act of infamy" and then declare war. So my last year or year and a half in high school, we were very much taken up with the war, and what we were going to do, and how we were going to participate. I had made up my mind that as soon as I turned of age, I was going to enlist in the navy. I graduated in 1943, so I was sixteen and a half. Almost immediately after I graduated, a friend of mine and I enlisted in the navy air corps, the navy flight program, and we were to be called up somewhere.

II COLLEGE, GRADUATE, AND POSTGRADUATE STUDENT

Chemical Engineering Student at City College of New York

Berg: In the meantime, I certainly had the ambition to go to college. My family wasn't well off enough to allow me to go wherever I wanted, so I went to City College in New York, which was free if you had the appropriate grades. And so I was admitted to City College. Now I can't understand why, but at that time I set out to be a chemical engineer. Quite different than all this medical ambition I grew up with. I don't know who prompted me to think that going into medicine was difficult for a Jewish guy at that stage. I know Kornberg himself had the same reservations, the same concerns, and the same warnings.[1] Chemical engineering was something that was practical, was something that you could easily count on as a career and for making a living.

Anyway, that whole thing at City College lasted three days. Because from where I lived, which was at the southern tip of Brooklyn, it took me, to go to City College in upper Manhattan, almost three hours to commute one way, two and a half the other. I was committed to being a chemical engineer, but I had already enlisted in the navy, and I knew that they were going to call me sometime.

The procedure at City College for registering for classes was about as baroque as anything you could possibly imagine. There was a certain number of courses that were available. And in one room of this immense hall, they had all the courses listed up on a board. As soon as a sufficient number of students had registered for that course, they took it off the board. You tried

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[1] For Kornberg's view on anti-Semitism, see Arthur Kornberg, *Biochemistry at Stanford and Biotechnology at DNAX*, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 1998.

to make up your schedule sitting in a room three hundred yards away from a printed schedule. After you made up a schedule, you got in a queue, and you walked through this line being badgered all the while by the ROTC [Reserve Officer Training Corps] people, trying to make sure that you had enlisted in the ROTC. And when you got to the tally, they would look up on the board, and if the course was closed that was it. So I spent two days of never getting a schedule.

Finally, for those that were left over, you took whatever leavings there were. And so, I would have a course at eight o'clock in the morning, and another one at one o'clock in the afternoon. Of course, coming from a place that was two and a half hours away, an eight-o'clock class meant I left home at the crack of dawn. So, I did that for three days, and I decided it wouldn't work.

The first class I went to was a physics class. Now, I had come from a high school which was not specially oriented towards science, certainly not to the extent of several special high schools in Brooklyn at that time, one of them called Brooklyn Tech, which was very much oriented towards people going into engineering or science. As high school students, they had already had many of the kinds of courses that anybody else would take when they went to a university.

In the first physics class I sat in on, we were given an exercise to make some measurements. They were trying to teach us something about the variables in making repetitive measurements and how to determine the significance of the measurements. There were these blocks of wood on the table, and some kind of a tool that I had never seen before, and a data pad. I was told to start making these measurements, and I didn't even know what this tool was or how to use it. I looked over at people sitting on either side of me, and they were going ahead and measuring. When I asked somebody if he could show me how to use it, I got brushed off very abruptly. So it was clear that the atmosphere there was very intense, highly competitive, and I would say certainly not friendly.

Biology Student at the Brooklyn College Campus

Berg: I didn't like the prospect of continuing that for I don't know how long, so I withdrew, and I went to another of the City Colleges, which is the Brooklyn College campus, much closer to home. I enrolled as a biology major, since I knew chemical engineering was

definitely not what I wanted. Especially since in one of the lectures, somebody talked about what chemical engineers do, which was building factories to manufacture various kinds of chemicals, and designing the machinery for carrying out certain processes. I realized that was not what I wanted. I actually was more interested in bench research. So, I decided I would go into biology.

Biology was exceedingly disappointing. It was the classical thing where you dissect some little pickled guinea pig or something, and then make exotic drawings of everything in it. Totally, totally boring. And so there I was, really disappointed. I didn't like the chemistry; I didn't like the biology--what to do?

Biochemistry Student at Pennsylvania State University, 1943-1948

Berg: At that time a friend of mine, who also lived in Seagate and was an engineering student, went off to Penn State. He sent me a catalog of Penn State, and I thumbed through it, and lo and behold, there was something called biochemistry, the Department of Biochemistry. This was not chemistry and it wasn't biology, but it looked like it was what I was really interested in the processes that go on in living organisms. So, I decided to go to Penn State.

Now that I think about it, I think I graduated in January, 1943, and attended Brooklyn College in the spring term. That summer, which would be the summer of 1943, I turned seventeen, and I was allowed to enlist. I went off to Penn State, waiting for the navy to call me.

Hughes: Was it a full department of biochemistry?

Berg: Yes.

Hughes: That was a bit unusual.

Berg: It was unusual. But it was not unusual, because it was in the Agriculture School, which is where biochemistry was largely centered in the thirties and forties. Most of the focus of biochemistry at that time was on analytical chemistry of biological materials. The people that came out of that program generally ended up in the pharmaceutical industry or in the food industry. In fact, while I was going to college, I was working

summers in food companies, in their research labs, doing what they call today food technology.

The other kind of biochemistry was largely in medical schools, and it was generally called physiological chemistry. But I didn't know this Department of Biochemistry was in the ag school. It didn't make any difference because within a few months I was called up in the navy. So, I completed one semester at Penn State as a freshman student majoring in biochemistry.

Military Service in World War II

Berg: It was the navy procedure to send you back to the university for further training. If you had already been to the university, you were permitted to continue in whatever you had been studying, and doing in addition the few courses that the navy wanted you to take, which were navigation and a few different engineering courses. If you had never been to the university before--

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Berg: --you were immediately sent to a place and put into an engineering curriculum. People generally stayed in these places for about a year, before they were to go on to the next stage of the flight training program.

Hughes: That was a stroke of luck.

Berg: That was a big stroke of luck. Originally, I was sent to Middlebury College in Vermont. But I knew that the usual procedure was that if you had already started at a university, and it had a navy unit, you were usually sent to the place where you had been. So, I wrote to the navy and asked them if I could be reassigned to Penn State, which I was. So when I went back, I was wearing a navy uniform, and I lived in a navy barracks on the campus. All the courses that I took were the same courses I would have taken had I been a civilian, and that was fortunate. I did that for three semesters, I guess it was, one entire calendar year.

By that time it would be late 1944; the navy decided that the attrition of navy pilots was far smaller than they had anticipated, and so many of us were transferred from the navy flight training program into what they call the deck officer school. I was sent to a midshipman school to be trained for ship duty. And that was in New York City out on Long Island Sound, a

place called Fort Schuyler. We were what people came to call the ninety-day wonders. You went for three months and you were commissioned as an ensign in the navy.

I was assigned to a sub chaser program. And I went off then to this place in Key West which trained officers for duty on submarine chasers. Those kinds of ships that I was on did either convoy duty, which was to protect maritime shipping, chasing submarines, or controlling landing ships during an invasion. So, I didn't get to do what I wanted to do, which was why I enlisted; I wanted to fly, but I ended up in the navy doing sea duty. I stayed in the navy until after Hiroshima. Then I spent one year bringing ships back from the Pacific and various parts. In the summer of '46, I was released from the navy.

Hughes: You were in the Pacific?

Berg: Yes, and also I was on ships that were protecting convoys in the Caribbean. I don't think that most people really ever appreciated how much American shipping was lost to the German submarines that were literally stationed off the East Coast and particularly the Caribbean, because there was a lot of traffic through the Gulf of Mexico. They devastated American shipping.

Our job was to try to reduce that devastation. By that time, of course, the navy had already broken some of the German naval code. They knew where the submarines were, and so it wasn't quite as devastating. Britain came very, very close to losing the war early on, because supplies to them almost never got there, it was so bad. Until they broke the naval code, which is a story in itself, they were on the verge of desperation. But once they broke the code, they knew where the German submarines were, and they were able to avoid them.

When the war ended, there were a lot of ships that had to be brought back from the Pacific. And so we brought them back, and then took them into what they call mothballing; storing them in various tributaries. And then they just covered them over with this protective covering to presumably preserve them. I was released, as I say, in the summer of '46, and went back to Penn State that fall.

Return to Penn State

Marriage

Berg: Although I had been in the navy for close to three years, I hadn't really lost that much time. I only had two years to finish college. And I did that, and finished with a major in biochemistry. I got married somewhere along the line, actually at the end of the junior year [1947], to somebody I had known for years and years, although she was not from where I grew up. Millie and I had worked together one summer in New York City in a company in which her father was one of the executives, and I was the office boy, and she was the mail clerk. [laughter] I was sixteen and she was fifteen.

Hughes: That's quite a story.

Berg: We kept in touch with each other while I was in the navy, and when I came back to New York, sometimes we'd get together. But after I got out of the navy, we established contact and then we were married the next year, in 1947. So we're coming up to our fiftieth wedding anniversary this fall.

Decision to Do Graduate Work in Biochemistry

Berg: Those two years at Penn State were rather interesting, because that's when I really started doing biochemistry in a serious way.

Hughes: Why?

Berg: First of all, I was a little older than I would have been had I just gone straight through. I was married now, and really much more focused on getting out of school. I knew I wanted to go on to graduate school. I didn't know where or how. I had decided that medicine was not what I wanted to do. I did want to do research.

At the end of my junior year, I worked for General Foods Corporation, over in Hoboken, New Jersey, in their research labs, doing just standard analytical work. The following year, I worked at the Lipton Tea Company in their research laboratories, also in Hoboken, again doing largely food technology.

Hughes: What effect did those summer jobs have?

Berg: Well, the summer jobs had one strong motivating force. You could see that the people who had bachelors degrees were the ones who were being told what to do. And the people who were telling them what to do were people with Ph.D.s. Very quickly that was the deciding point, that if I was going to go into science, I did not want to be in a position where people were telling me what to do. I wanted to be able to drive the research myself. And that's when I decided that I wanted to do a Ph.D. So it was the end of the junior year, when I knew I was going to go on to graduate school.

My wife was a registered nurse. In fact, during the war she had been in nurses training. And when she graduated in 1947, we were married. She went back to State College, which is where Penn State is. State College was a town of about three thousand people, a dinky little place--no hospital. We were living in a rented room in somebody's rooming house. She tried to find a job, and a hospital was twelve miles away. We had no car. She obviously couldn't do that. So she worked for a while looking after newborn babies born to student wives during the first weeks after birth. That was clearly not satisfying. So she went back to New York City, lived with my parents, and worked in a local hospital there. So for that last year, we were separated. I used to commute back and forth from State College, about an eight-hour train trip, to New York for the weekends and then go back on Sunday nights. And that was a hassle.

During my last year, again, my performance in school was pretty good. I was identified pretty quickly as a bright young student to be helped. I wrote several papers, not for publication, but during my last year, 1948. One of them was about the newly emerging use of isotopes for tracing metabolic reactions, and that excited the heck out of me. I gave several talks on it.

Graduate Student, Western Reserve University, 1948-1952

Applying to Graduate Schools

Berg: So I applied to this one school, which seemed to be the place from which many of the review articles and published papers were coming. It was Western Reserve University, a place I had never heard of. I thought it was an Indian reservation. I wrote to

them, and they didn't have an opening for a new research assistant, a graduate student.

Hughes: Who was there?

Berg: A man named Harland Wood.

Hughes: He was the one using the isotopes?

Berg: He had just created a new department. I'll come back to that in a little bit, because it's a very important part of my life.

During that last year, knowing that I wanted to go to graduate school, I approached it in my typical way, which is, I got a book about all the graduate schools that had biochemistry departments, and I wrote a letter to each one of them applying for admission. I must have sent out about sixty letters.

It was the same way I got the summer jobs. I would write to every chemical, biology, drug company, food company in the New York Metropolitan area, and just wait for an answer. [laughter] And I always got an answer offering me a job for the summer. And so I did the same thing for graduate school, just applying to a large number of places. Not unexpectedly, some of them had biochemistry departments in the medical school, others in the agriculture school.

So, I applied to Western Reserve; it was one of the many that I applied to. It's an interesting fact relating to Kornberg's talk about anti-Semitism: I'd get back some letters which were offering me admission into the graduate program in biochemistry, with a warning or statement telling me that by no means should I consider this as a possibility that I would be able to enter the medical school through the back door. They were really quite up front about that.

Eventually, I chose to go to a place called Oklahoma A and M, which today is called Oklahoma State University. For a guy from Brooklyn to choose to go to Oklahoma A and M was already pretty radical. [laughter] But part of the reason was the department of chemistry offered us a place to live, found a job for my wife to work in the local hospital, and they were really recruiting heavily. So I agreed to go.

Hughes: You went for those somewhat peripheral reasons, not for the science?

Berg: That's right. It was known as a pretty good school of chemistry, but I didn't know a lot more about it. And so I accepted.

Decision to Attend Western Reserve University

Berg: Within about two weeks of the time we were going to leave Penn State, and planning to go west, I got a call or letter from Western Reserve saying that they now had an opening, and they had money, and if I would like to come, it would be worked out. I was very, very troubled because I had already accepted this very generous offer from Oklahoma A and M, and I remember being very troubled about turning it down.

I went to see both the dean and the chairman of the biochemistry department at Penn State, and to this day, I can never thank them enough for having said, "Look, just call up Oklahoma A and M and tell them straight off that you've had your mind set on doing this kind of work, and here was an opportunity to do that." I did that and they were extraordinarily gracious. I was so bowled over, because it was so unexpected. I thought they would give me a hard time by saying how much difficulty they had gone through to get me settled.

The Department of Clinical Biochemistry

Berg: In any case, when I got to Western Reserve in Cleveland in 1948, what I found is that I had been accepted by the wrong department. [laughter] In fact, what I had actually applied to was an ad in the *Chemical and Engineering News*, in the back section where they printed job openings. And it said, "Western Reserve University, Department of Clinical Biochemistry, Medical School." And since I recognized that Western Reserve University was the place from which all these papers were coming that I was so excited about, I just assumed that they were one and the same.

The biochemistry department at Western Reserve University had been headed by a man, Victor Meyers, since 1915 or '20, who by 1948 was literally senile and long since beyond any period when he had been productive. When the war ended, Western Reserve University realized they had to rejuvenate the biochemistry department. So they recruited from Iowa what was then one of the hottest young biochemists in the country, named Harland Wood. He brought his entire group from Iowa, and several people who were in Minnesota. He created a department essentially in one fell swoop. What to do with the man who had been head of the department? They created Clinical Biochemistry, of which Victor Meyers was the sole occupant.

So, when I got there, I was told I was going to be in this department, and I realized that it had nothing to do with the one I wanted, which was on the floor above. But there wasn't much I could do; I was there. And when I went to see Victor Meyers to discuss what I was going to do, he gave me a couple of old theses, which were by people who had analyzed cholesterol levels in various tissues of autopsied individuals. And my assignment was to do the cholesterol level in some eighty-five postmortem heart muscles and see if I could correlate cholesterol level with cause of death. Well, that was pretty discouraging.

Research on the Artificial Kidney

Berg: Fortunately, Meyers died about a month later, and so there I was stuck in this pseudo-department. But two of Meyers' earlier students were working in the lab, and one of them, Jack Leonards, had been given a junior appointment in the department of biochemistry, largely as a courtesy. He asked me if I would be willing to stay on and work with him. Well, I didn't have much choice, so I said yes.

It turned out that he and this other person, Leonard Skaggs, who had also been a former student there, were developing an artificial kidney. At that time there was already a precursor, which had been developed by a Dutchman named Kolff, and which was in use, but was very, very complicated. It was a big rotating drum around which dialyzing tubing was wrapped, and blood ran through the tubing, and this drum rotated it through a bath which allowed dialysis to occur. So toxic substances that were in the blood could pass through the membrane into this bath.

These two guys had come up with a design which was sort of a neat little box. It was a very ingenious idea. And so I said, I have nothing to lose; I'd work for them. So I started to use the artificial kidney to learn how to keep animals alive after you've taken their kidneys out. I had to learn a lot of surgery and things I wasn't terribly interested in.

Joining the Department of Biochemistry

Berg: Meanwhile, I was taking courses that graduate students had to take. All the courses were in the biochemistry department, plus the first two years of medical school courses. Well, one of the

courses I had to take up in the biochemistry department was a course in which students were asked to give presentations on current research papers, and I gave one or two of them. And not long afterwards, I was approached by the chairman of the department: Would I consider moving up into the biochemistry department? Well, that was the kind of thing you'd say, "I thought you'd never ask."

So, at that point, I went to these two fellows and told them that I had always had my heart set on working in this lab where there was use of isotopes, and not on this type of physiological chemistry that they were doing. They were very good about saying, "By all means, go." And so I moved up to the department and became a graduate student there.

I had already spent two years in this process and at that time a Ph.D. program was normally four years. People did not take longer. And so I essentially did my thesis research in two years. Our students today take five, six years and don't do half what we did, because we had to teach, and we had to run the laboratories for the medical students and take a lot of courses. We had to take almost the entire curriculum for the first two years of medical school, even though we were not going to go on and be doctors.

Harland Wood and the people in the department were different role models than anything I had seen. First of all, it was an academic setting. It wasn't an industrial lab. And the pace of discovery in my research went extremely well. I received a lot of kudos and gave talks at national meetings, and so on. The feedback I got was that I could do this; this is a career that I could be successful in. And it looked like it was great fun, to just go into a lab and be able to do whatever you wanted. Teaching was not a difficult thing, and jobs were plentiful.

Hughes: So, you were teaching while you were learning.

Berg: That's right.

Hughes: You hadn't had much formal biochemistry, had you?

Berg: Well, what we did was teach in the laboratories. Biochemistry courses had laboratories associated with them. Medical students had to go in and carry out various exercises. There were maybe twenty or thirty experiments over a term, and you were there to help them. We had to set up the reagents for them. We had to test the experiments to see if they would work, and things of that sort. And we had to spend long hours in the laboratory with the students. And then when the laboratory was over, we could go and

work. The general pattern of working for a graduate student is essentially into the wee hours of the morning.

My wife was a nurse at Western Reserve Medical Center, a big hospital which was part of the medical center. And she chose to work some very interesting hours, like six to midnight. That meant she had the day free, and I could go back in the evening and work until midnight. And then I'd go pick her up from the hospital, and then we'd go do something sometimes. That was a very good thing, because the hospital had a hard time getting people to work those hours, so she only had to work six hours and was paid for eight, which was nice. She largely supported the two of us. I was on the G.I. Bill, which I think was something like $165 a month. I don't know, her salary was maybe twice that. And so we lived on that.

We had an apartment very near the medical center, so we could both walk to the lab or to the hospital. We spent four years in Cleveland, which actually was great fun. We really enjoyed it, made a lot of friends. The principal thing about it was, it opened up new vistas for what I wanted to do.

Research on Nutritional Supplements

Hughes: What was the research?

Berg: It was to solve a problem that had really been a central theme or problem in biochemistry. Animals on certain diets, for reasons that nobody could tell, would die. Well, that's not a very good explanation. There were certain compounds or nutrients that were thought to be an essential part of every person's diet. And if you left them out of the diet, they led to death. One was the amino acid, methionine, and a compound called choline. If methionine was omitted from the diet, animals usually developed very fatty livers and then ultimately died.

About the time that I was plugging away at dialyzing animals, one of the great figures in biochemistry, a man by the name of Vincent du Vigneaud, was a professor of biochemistry at Cornell Medical School, and later received the Nobel Prize [1953]. He was one of the ones that had reported that methionine was an essential part of the diet. But there were hints that this wasn't necessarily always true, because if you varied the diet, supplemented it with certain vitamins, you could leave methionine out, and the animals did fine. The two things were folic acid and vitamin B-12. When you looked in the tissue in the diet, what you

found is that the animals contained methionine and choline which previously had to be supplied in the diet. So, it was obvious that given these vitamin supplements, they were able to completely synthesize these molecules, which had been thought to be unable to be synthesized.

There were already some indications of what kind of molecules could be used as the building blocks to build methionine and choline. One of the people, Warwick Sakami, in the biochemistry department where I was had done an experiment injecting radioactive formaldehyde into rats and then recovering methionine and choline from their livers. He showed, using this radioactive formaldehyde, that the methyl groups of methionine and choline had been produced using the formaldehyde.

One of the great, great things about using radioisotope tracers was that you could feed a molecule that was marked radioactive, and you could determine whatever it ended up in, the new molecule because it contained radioactivity. So, formaldehyde had been converted into the methyl groups of methionine and choline. And so, it looked like these animals, when provided with the appropriate vitamins, were actually able to convert the one-carbon compound, formaldehyde, and subsequently formic acid and methyl alcohol. All three of these, in fact, could eventually be converted in the body to the methyl groups of methionine and choline.

Berg's Initial Research Project

Berg: In one of the seminars, when I was still a student in Clinical Biochemistry, I reported this progress. And in preparing the seminars, it came to me that there were certain kinds of experiments that could be done that would test how this synthesis went. And so, I went to Warwick Sakami, in biochemistry, and I told him that it seemed to me that one could find out something about this process, by the following kind of experiment. And that's when he said, "How would you like to come up here to the biochemistry department and do it?" And then he went to the chairman of the department, and Harland Wood then asked me if I would be interested in coming up. So, when I went up, I did this experiment, and it came out beautifully, describing for the first time how this one-carbon molecule could get into these other products.

Hughes: So they were intrigued by the science that you proposed to do. But were they also impressed by the fact that you were using a

technique that Harland Wood was interested in, namely the use of radioisotopes?

Berg: Everybody in the department was doing that.

Hughes: Using radioisotopes.

Berg: Using radioisotopes. So, I didn't bring anything novel to the idea of using radioisotopes. It was more how to use the technology to actually explain an interesting and at that time unknown biological process. So my recollection of what they said is, "That's a neat idea. Why don't you do it?" So, I went up and set up the experiments and actually started to do them. And as I say, they came out pretty much the way I predicted.

That then led me to try and do enzyme experiments, asking could we demonstrate the enzymes that carry out this process? Well, at that time, I wasn't very much of an enzymologist. All I could do was set up these tissue extracts. They were relatively crude extracts. But we showed we could convert formic acid or formaldehyde to the methyl groups of methionine and choline in a cell-free system.

Hughes: Was that something that people with a biochemical background would have known how to do?

Berg: You mean how to do the extracts?

Hughes: Yes.

Berg: Yes, doing that was not so novel. Kornberg was doing enzymology long before that. People were studying how one-carbon compounds are used to make purines in nucleic acids. So, what I did was actually set up for the first time an in vitro system that could actually manufacture methionine and choline, and I used these radioactively tagged molecules to show how that conversion went. I was able to show that in fact in these extracts there was evidence that you needed cofactors, so-called, which were derived from folic acid, which had been hinted at by showing that animals fed high levels of folic acids and B-12 could make everything. So, it was not terribly surprising. I published several papers on that.[1]

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[1] For references throughout the oral history to Dr. Berg's publications, see his bibliography in the appendix.

Countering du Vigneaud

Berg: One of the things that happened was, I went to a national meeting, and when I presented this work, Professor du Vigneaud, this man who had been the real honcho for this whole field, got up and gave me a hard time. He had been protecting the principle and concept that these substances were required in the diet. And here was somebody getting up and showing that they were not only not required, but here was a way in which they could be made in the body.

I must have handled myself very well in this debate, because plenty of people told me about it afterwards. But interestingly enough, du Vigneaud went to Harland Wood and said, "You have a young professor in your department, Paul Berg. I want to offer him a job here at Cornell in New York." And Wood told him, "He's not a professor; he's a graduate student." [laughter] So, my work really flourished; it went very well. And by the time I was ready to go, I was already known somewhat outside Western Reserve.

Harland Wood and Radioisotopic Tracers

Berg: Now, there was a steady parade of distinguished people who came through Western Reserve because Harland Wood was one of the very leading top people in this field. He had developed whole new approaches to studying metabolism using these isotopes. And he had invented instruments that made it possible to study both radioactive and stable isotopes--stable isotopes like carbon-13. He had built a mass spectrometer to do this kind of analysis. He was a remarkable guy.

Hughes: Postwar, radioisotopes were relatively easy to obtain?

Berg: Easy to obtain because Oak Ridge Laboratory was cranking them out, and they were commercially available. Radioactive isotopes carbon-14 and P [phosphorus]-32 were available. The other isotopes were much harder to get, but they too could be obtained, because they were made by some form of enrichment. Carbon-13 is normally present in carbon dioxide. But it's present in only small quantities, and nitrogen-15 is present in all kinds of nitrogen-containing compounds, again, in small quantities. During the war they learned how to enrich for these heavy isotopes, as they were called. And the radioactive isotopes were made in a radiation lab.

During the war, before radioisotopes were available, Harland Wood was restricted to using carbon-13, and he did one of the great, monumental pieces of work in discovering that carbon dioxide is actually metabolized. We build lots of our organic molecules from carbon dioxide that we had always considered as a waste product.

If you label the carbon dioxide and feed it to animals, you can show that they in fact make many complex molecules from the carbon dioxide. But he didn't have Geiger counters to measure because this was a stable isotope. He had to develop a mass spectrometer that measures the mass of atoms, so you could distinguish carbon-13 from carbon-12, which are the natural carbon isotopes.

Wood was one of these people raised on a farm for whom building anything was not an impediment to doing what you wanted to do. He built anything. He synthesized all his radioactive compounds. He was a really remarkable guy, and he was a wonderful person so that you were strongly attracted to him. What he represented was an idea of what you would love to be like. That's why most of the students adored him. In fact, I have a visit next week from a man who is writing his biography.

We remained very close friends for many, many years. He died about five, eight years ago. But he continued working into his eighties. In the last decade of his life, he probably published more papers than the rest of the department together. It was amazing. We went to his seventieth birthday and his eightieth birthday.

Hughes: You were using radioisotopes mainly, rather than stable isotopes?

Berg: Yes.

Hughes: And getting them from Oak Ridge?

Berg: Yes, you could buy them. You bought barium carbonate, which was an insoluble compound. It's essentially a barium salt of carbonate, $BaCO_3$. Organic chemists had worked out techniques that converted barium carbonate to sodium bicarbonate, and then doing organic chemistry to convert the sodium bicarbonate into organic molecules. And we had to do all that.

Today, students just go to the catalogs and buy everything; they make nothing. But we had to synthesize everything that we made. And so a lot of times we had to learn the chemistry to make methyl alcohol, how do you make propionic acid, or pyruvic acid, or any of these molecules with a radioactive carbon atom in a

particular location in the molecule? These were fairly complicated steps.

Hughes: Was that expertise in the department?

Berg: Yes, that was expertise that was largely in the department, and we got lots of help. Sometimes you had to work out a procedure that had not been done before. So you made your precursor molecules. You either fed them to animals or injected them into animals or incubated them with extracts. And then you recovered the products, and then you had to determine if the product was radioactive. That was easy. And then you had to ask where did this radioactive atom end up in the molecule? So we had to develop procedures for degrading molecules and recovering the different pieces in a form where you could measure their radioactivity. And then you could draw the chemical reactions that must have intervened to convert this into that, if this molecule ended up in that particular part of the other molecule. And so we had to develop procedures for degrading molecules and recovering the different pieces in a form where you could measure their radioactivity.

Radioactivity made this kind of experimentation much simpler than using stable isotopes. With stable isotopes, you had to have this very elaborate mass spectrometer, and you had to recover larger amounts of material. Whereas with radioactivity, you could use literally trace quantities.

I remember very clearly once having to make methyl alcohol from barium carbonate. I did this in a very large hood, with ten millicuries of radioactivity, which was a very, very large amount of radioactivity at that time. Today, people wouldn't think it was very much. I set up this whole organic synthesis array, in which the starting material was here, and the products had to go through various kinds of bubblers and be collected in a reservoir. I had to build the glassware myself. And when I did the experiment, and I looked at the end, I had nothing. It turned out that one of the glass bubblers had a pinhole in it, and all of the gas had gone through the hole. I had lost ten millicuries of carbon-14, for which I was ridiculed and got a lot of flack. But that was the trial and tribulation of learning. [laughter]

Visits by Arthur Kornberg and Herman Kalckar

Berg: I remember very clearly, Arthur Kornberg came to visit Western Reserve University and gave a lecture. It was not long after he

had won the Paul-Lewis Award [1951] for his work in enzymes. This would be about 1951 or '52. His picture had been on the cover of *Chemical and Engineering News*. At that time, even small prizes got you a lot of notoriety. Today, you win a prize, you might earn a little paragraph in the back of the journal.

But anyway, when I read about Arthur, I discovered that he was from Abraham Lincoln High School, grew up in Brooklyn, and so on. And so after he gave his lecture, Harland Wood had a cocktail party at his home for him, and I was invited, and he and I chatted. We discovered that we were both from the same high school and the same background. I asked him if there was a chance that I could come work in his lab as a postdoc.

Hughes: You knew what research he was doing because you had gone to the lecture?

Berg: Oh yes, not only his lecture but during graduate training we studied every new paper that came out, and so I knew in great detail what he had been doing. It was the kind of enzymology which I had not had experience with, nor was there anybody in the department at the time who did that kind of enzymology.

Hughes: Which was what?

Berg: Well, fractionation, obtaining pure enzymes. We were content to work with relatively crude preparations. Kornberg would turn over in his grave if he knew any of his people ever worked with crude extracts.

Hughes: Was he fairly unique at that time in insisting on working with pure extracts?

Berg: Well, I think he was one of a small number. There were perhaps half a dozen people who were really committed, and one of them made the point, "Don't waste clean thoughts on dirty enzymes." Kornberg had taken a sabbatical and gone to work with a man named Carl Cori, who was one of the giants of biochemistry.

Berg: There were lots of other visitors, and one of them was a man who was from Denmark; his name was Herman Kalckar. Herman Kalckar was a brilliant scientist and had made major discoveries during the late thirties, when he was a student in Copenhagen. And then he came to this country on a Rockefellar fellowship. He went to Caltech, and then the war broke out in 1939, and he was stuck in the States. He remained at Caltech for a short time and wrote an extremely influential review article, which gave him a lot of notoriety. He then took a position at the Public Health Research Institute in New York, and he spent the war years there. He

developed a whole new approach to being able to use enzymes in a novel way.

Herman came and gave a seminar on that topic. I don't know how many Danish people you know, but many of them are unintelligible. He was more unintelligible than most. Their language requires a lot of guttural sounds and throat sounds. When Herman spoke English, he was very hard to understand, and he had a flamboyant way of doing it that made it even more difficult. So, nobody in the audience knew what he was talking about. But I loved him; I thought he was great.

Hughes: Did you understand what he was saying?

Berg: I could see the outlines of what he had done, and it was fascinating, because it looked like it was a new way of using enzymology. What he was really developing was an analytical way to follow enzyme reactions.

Hughes: But not with radioisotopes?

Berg: Not with isotopes. This was using a spectrophotometer. You get spectral changes in certain molecules when the enzyme acts on them, and therefore you can follow the reaction by following the spectral changes. At that time there was a very new instrument that had come out called the Beckman spectrophotometer. And the Beckman spectrophotometer was one of the special instruments that everybody sort of bowed around. And here Kalckar was using this thing in this very clever way, and it just struck me. Besides, he seemed like an interesting personality. When the war ended, he of course went back to Copenhagen. So, I talked to him about the possibility of going to work in his lab, and he said that was fine.

Postdoctoral Positions

Deciding Not to Go to the Coris' Lab

Berg: Meanwhile, Harland Wood had other plans for me. He had seen me as one of his star students, and he felt that it was appropriate for me to go on to Carl Cori's lab, and that it was a fitting place for a bright young person to go and get the next level of his training. Carl Cori was at Washington University in St. Louis, and the stories I heard about St. Louis made it sound like it was unlivable.

Hughes: The city itself?

Berg: Well, its location. It was known for temperatures in excess of a hundred degrees Fahrenheit during the summer, maybe four or five months of the year. And it was a southern city still. It was relatively racist. I think blacks still had to sit in the back of the bus at that time. It was very unappetizing. And so, I told Harland Wood that I would not go to Cori's lab; I did not want to live in St. Louis. He was greatly disappointed by anybody who would turn down an opportunity to work with the great Carl Cori.

Hughes: You appreciated Cori's stature?

Berg: Oh, I knew Cori by name, and I had seen him at meetings. There was no question that he and his wife, Gerty Cori, both of whom had gotten the Nobel Prize, were sort of the top people in the field. Arthur Kornberg had gone to do his apprenticeship there and came to revere Carl Cori as well. So I lost a lot of stature with Harland Wood by saying I wasn't going to work with Carl Cori. I was going to work with Herman Kalckar in Copenhagen for a year [1952-1953], and then I would spend a second year [1953-1954] with Arthur Kornberg at the NIH. And that's what I did. So, we went off to Copenhagen; I spent a year.

Postdoctoral Research Fellow with Arthur Kornberg, Washington University, 1953-1954

Berg: During the time I was in Copenhagen, Arthur Kornberg was invited to become the professor of microbiology at Washington University in St. Louis. And so he wrote to me and said he hoped I still planned to join him when he moved and took over this new department. And I agreed to do it. So, I ended up in St. Louis anyway. [laughter]

Hughes: Now, why did you agree the second time?

Berg: Because the prospect of working with Kornberg seemed much more appealing than working with Cori. Cori was a great figure; Arthur was a rising young star. And the kind of enzymology he was doing was more appealing to me. He was much more aggressive in wanting me to come than anything I had ever heard from Cori. Cori would have taken me largely because Harland Wood would have said, "I want you to take Paul Berg on." Whereas Arthur really wanted me to come.

Arthur had gotten enough about me from other people. I kept in touch with him when I was in Copenhagen, telling him about the experiments we were doing. He was very much taken by the discovery we made, and so he wanted me to come. Also he was moving into a new venture. He had always had a sheltered life being at the NIH, and here he was now taking an academic position. He had the job of creating a whole new department. I'm sure he must have thought a little bit about what kinds of people he wanted to recruit. At least he had a chance to have a look-see at me as a postdoc. And as it turned out he eventually offered me a position in the department.

Postdoctoral Research Fellow with Herman Kalckar, Institute of Cytophysiology, Copenhagen, 1952-1953

Berg: Copenhagen was really great because working with Kalckar was great fun. I learned to understand him, and if I didn't understand him, I learned not to be embarrassed to just keep plugging away. What I found was that the easiest way was to take him to a chalkboard or blackboard, and we'd write what we had to communicate. If I didn't understand him, we would try to draw the experiment. After a while I got to be able not only to follow him, but almost to anticipate the kinds of things he would be thinking about.

Hughes: I think of [Niels] Bohr--

Berg: Bohr was almost as unintelligible. In fact, I'll tell you a little story. While I was in Copenhagen, Herman Kalckar was invited to speak at the Danish Royal Academy of Sciences. He took me and one of the other people in the lab along as his guests. It was a rather spectacular Old World setting, with enormous paintings on the wall of great figures in Scandinavian science.

There were two talks scheduled, followed by Danish sandwiches and good beer. And the first speaker was an elderly man. In fact, most of the members were extremely old. They all had earphones, because most couldn't hear. I couldn't understand the first speaker; he was speaking Danish. It turned out he was a linguist, who gave a talk on some of the most erotic and pornographic passages in Danish poetry. And so there was a lot of snickering, and so on and so forth.

Hughes: You missed it all. [laughter]

Berg: And I missed it all. I was sitting, looking, and staring around at the pictures. Then Kalckar got up, and he started to speak.

He was giving a scientific lecture about the research. And you could see within three minutes, five minutes, the earphones came off all these guys, and they sat back and just either slept or whatever. [laughter]

Bohr, who was there, fell asleep. And when he did speak, he was, at least to me, unintelligible. I gathered that even in Danish, Kalckar was hard to understand. So when he was asked to give lectures to the medical students, they didn't invite him back again after he would give the first couple. He had an unusual way of mumbling, which is not uncommon amongst Danes. And Bohr mumbled also. Danes refer to their language as a throat disease, not a language, plus this mumbling. It was just very hard. And in English, it wasn't a heck of a lot easier.

Nevertheless, we had a great group in the lab. It was a group from all over the world--from India, from Canada, from Italy, and I forget where else, plus Danes, Swedes. It was a very coherent, friendly group. It was an exciting period, because the work that was going on in the lab was booming. It was one of these things where a new discovery had opened up a whole new world, and everything you did was new and successful.

Discovery of Nucleoside Diphosphokinase

Berg: I got involved in a project which very quickly turned out to be hugely successful. An Australian postdoc Bill Joklik and I worked together and published two or three papers on this one discovery for one year's work.

Hughes: Did you come with an idea for a research project?

Berg: No. Kalckar had a physicist friend Thomas Rosenberg who dabbled in biology and had some kind of cockeyed idea to explain a particular reaction involving hexokinase. It's an enzyme that converts glucose into a phosphorylated form of glucose, and it's a very key step in the metabolism of sugars. It is normally viewed as an irreversible reaction. It's a reaction between glucose and ATP [adenosine triphosphate] which forms a compound called glucose-6-phosphate and ADP [adenosine diphosphate], and was not known to be reversible.

He postulated that the reaction went in two steps; that an intermediate, which was very, very reactive, immediately went on to the product that everybody knew. This intermediate product, he supposed, was a very special form of glucose phosphate, so-called

high energy, he called it. And so we talked about this hypothesis; there was no evidence for it. This was a reaction that Carl Cori had studied in great detail and had found evidence that there might be a step that was influenced by insulin.

Rosenberg's idea was that insulin involved the second step of this two-step reaction. So, I looked at it and listened. And I said, "If that is true, one ought to be able to do the following kind of experiment and show that you could transfer P_{32} from ATP through this hypothetical intermediate to another molecule like ATP, ITP. It was known ATP would not transfer phosphate to ITP.

So, Kalckar said, "That's an interesting idea. Why don't you try and do that?" It was known that there was another form of ATP, called ITP, which could react with glucose. So, I came to the conclusion that if we took ATP with P_{32}, and if we phosphorylated glucose to this glucose metaphosphate, as we called it, that glucose metaphosphate could transfer the P_{32} back to ATP, but it could also transfer it to ITP. So, in other words, you could transfer phosphate from ATP to ITP, but it would require glucose.

So, we set up this experiment. We had to make our own radioactive ATP. The idea was to put in radioactive ATP and study whether the P_{32} transferred to ITP and required the presence of glucose and the enzymes. Well, the experiment worked beautifully--P_{32} went from ATP to ITP like gangbusters, but it didn't require glucose.

What we had discovered was a new reaction in which ATP can transfer phosphate to molecules like ITP, and GTP, and CTP, and UTP. It doesn't have anything to do with glucose; it doesn't have anything to do with hexokinase. It was a wholly new enzyme. So, just by setting up an experiment to test a particular hypothesis, you test one kind of question and you find something completely different, which is often true in science.

Making Radioactive ATP

Berg: There were companies that were making radioactive ATP, but dollars were needed to pay, and Denmark didn't have dollars. So, we had to make it ourselves. The traditional way of making P_{32}-labelled ATP was to inject a lot of P_{32} phosphate into a rabbit, exercise the rabbit so that it breaks down all its muscle ATP, and then allow the rabbit to recover and rebuild its ATP. Then you kill

the animal and you can dissect its muscles, and after a whole series of extractions and a lot of complicated steps you get ATP.

We had an old bathtub up in the attic of the institute. And so we decided we were going to inject this rabbit with about, I don't know, twenty millicuries of P_{32}, which was a lot. We put this rabbit into the bathtub, expecting he was going to swim, but he just sank to the bottom. We kept rescuing him and saying, "Swim, you bastard." [laughter] We kept dunking this rabbit, and he just wouldn't swim.

But eventually we got him out, and he was shivering. And we figured that was enough. So we let him shiver for a while, and then we wrapped him in a towel and put him near a radiator and warmed him up for a while. Then we sacrificed him. Then we cut him up and isolated all his muscle and eventually isolated a lot of ATP. And that's what we used. It was such a funny story. I tell that to students today, and they just can't imagine that anybody ever made their own ATP.

Life in Denmark

Berg: So, things went extremely well in Copenhagen. We had a great time. We met a lot of wonderful people. We traveled a lot, all through Europe. We went skiing in Norway, visited Sweden, traveled through Germany and Italy and the lakes region. It was very inexpensive. I was living on a fellowship which paid $3,500 a year. We literally saved half of it. We had a villa in this little fishing village outside of Copenhagen, and we saved enough money to do all this travel.

In fact, Danish friends told us we were making more than the King of Denmark, in terms of our income in dollars. And we ate extremely well, even though Copenhagen was just emerging from the effects of the Occupation. The war was over in 1946; in 1952, there was still rationing when we got there. I remember going to George Jensen's silver shop in the town, and everybody gathered around the window looking, not at the silver bowl, but at the peaches it held.

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Berg: We couldn't buy certain kinds of food. Meat was rationed. By the time we left, the rationing was just coming off. I remember we didn't have a refrigerator in this little house; we had a little door to the outside of the house so milk could be delivered. The

milkman would open the door, put the milk inside. It was open to the outside temperatures; it kept things cooler. Eventually we prevailed on the woman who owned the house to get us a small refrigerator.

We loved living there. We lived in this village, which was on the edge of this royal deer park. So when we had to walk to the train, which took me into work every day, we walked along with deer, and during the mating season you could hear this roaring. It was incredible.

James D. Watson

Hughes: How common was it then for American graduate students to go abroad?

Berg: It was just becoming both fashionable and possible, largely because of the postdoctoral fellowship program that the NIH, the American Cancer Society, a number of them had. Jim Watson, for example, went to work with Herman Kalckar.

Hughes: Was that the same year?

Berg: Year before [1951]. When Jim went, Kalckar was not ever around. Kalckar had a postdoc whose name was Barbara Wright, and he and Barbara took up with each other. He left his wife, and he and Barbara traveled all over Europe together. And so Jim never saw Kalckar. He went to work with Kalckar to learn something about enzymes. His advisor [Salvador] Luria said to him, "You ought to learn some chemistry." And so Jim went there, but Kalckar wasn't there. So, he moved to work with another man named Ole Maaloe who was working at the State Serum Institute on bacteriophage. And to this day, and on many, many occasions, Jim will talk about this lost year in Copenhagen and about Kalckar.

I came the year after. By that time Kalckar and his wife had separated, and he married Barbara. And so I got to know Barbara. They had a child, which I guess was conceived well before they were married. We had a great time together. So, for me, that year was very successful. It was such a bad experience for Watson that he left Maaloe after the year and went to Cambridge.

Hughes: Just think how history would have been different if he had had a successful year with Kalckar.

Berg: He might never have gone to Cambridge. In fact, Watson tells the story that from Copenhagen he went to a meeting in Italy where people were talking for the first time about DNA as the genetic material. There was some preliminary structure work. And he got all turned on about the need to solve the structure of DNA. He left Copenhagen and got into terrible trouble with the American Cancer Society, because they had given him the fellowship to work in Kalckar's lab, and he didn't even notify them; he just moved to Cambridge.

I remember Luria telling me that he had to write all these letters to the American Cancer Society, trying to get them to accept that Watson was a little bit bizarre and to forgive him. But he was better off in Cambridge. And it was in Cambridge that he and Francis [Crick] did that work on DNA structure.

Hughes: So, he went to Kalckar's lab not with DNA on his mind at all.

Berg: No, not at all. He wrote about that period in his book about the discovery of the double helix.[1]

More on the Postdoc with Kornberg

Institutional Setting

Berg: After Copenhagen, I came back to St. Louis.

Hughes: Where Kornberg was already ensconced.[2]

Berg: He was already there. He got there in January 1953; we got there in October. And the lab was very primitive. It had been an old clinic; in fact it was a clinic building. The first floor was where indigent people would be sitting or sprawled in chairs for hours, waiting to see the doctor. There was this rickety elevator that took you up to the fourth floor. And there was the microbiology department, which literally had rooms with bare bulbs hanging from the roof. It was just an amazing thing.

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[1] James D. Watson, *The Double Helix: A Personal Account of the Discovery of the Structure of DNA*, New York: Signet Books, 1968.

[2] For Kornberg's views on his time at Washington University, see his oral history, cited on page 8.

Kornberg took this position because he really wanted to leave the NIH. Cori had been a very strong force in persuading him to come to Washington University. Besides, Washington University was already well known; it was a place that had a strong tradition in research. I think Kornberg must have been promised a whole lot of support. [phone interruption]

Arthur tells the story that the then dean of the medical school, a man named Oliver Lowry, who was a very well-known pharmacologist, was head of the pharmacology department. He was a tinkerer. He told Arthur that he would come up and personally paint the labs so that they were presentable. He was going to help with some of the wiring and fix up the place. He never did. But he was that kind of a person anyway. It was so amusing.

When I got there, probably there were two or three labs that were fixed up. One was the one that Arthur and his wife [Sylvy] were working in, with a couple of postdoctoral fellows. Another one was a very large lab which could probably have held six or eight people, in which there was nobody working but had been fixed up. Painted, lit, it looked presentable as a lab. And two other labs, to which he brought people who had been postdoctoral fellows at the NIH, and to whom he gave appointments as professors in the new department.

Hughes: And who were they?

Berg: One was named Osamu Hayaishi, who went on to become one of the leading biochemists in Japan and today in his eighties is probably one of the most influential people in science in Japan. The other was a man named Irving Lieberman, who eventually left never to be heard of again. I don't know what ever happened to him. He was extremely able in the lab, but kind of a weird guy. And the two of them never got on very well, so he left.

When I came, Kornberg took me into this nice new lab and said, "Here's where you're going to work." And I looked around at this decrepit place. He was telling me, "Don't worry. We're going to get it all fixed up." And we did over the years. I got my Ph.D. in '52, went abroad for the year, came back from Copenhagen in '53, and immediately we talked about what I might work on. Kornberg gave me several suggestions of projects.

Lipmann and Lynen's Hypothesis

Berg: While I was in Copenhagen there had been a very significant paper published from Fritz Lipmann's laboratory--Lipmann was one of the giants of biochemistry at the time--by a man by the name of Feodor Lynen, who was a professor in Munich. I can't remember whether Lipmann and Lynen had gotten the Nobel Prize by then, or they went on to get it.[1]

Lipmann and Lynen and two of their students had published a paper which provided a hypothesis or model for how a very key reaction in metabolism worked. This was a reaction that was very central [acetyl-CoA formation]; many, many people had been studying it. It was a rather complicated reaction. Lipmann and Lienen proposed a novel mechanism. A feature of the mechanism that they proposed was that the first step was a reaction between ATP and the enzyme, to knock off two phosphates from the ATP and make an AMP enzyme complex. And then there was a second step in which the AMP was transferred to coenzyme A, another one of the components.

Berg's Research on Acyl Adenylates

Berg: I was intrigued by this notion of an enzyme intermediate linked with the enzyme AMP. And I was intrigued by it because although I was interested in nucleic acids, I didn't really know anything about them. But it seemed conceivable that this form of AMP was an activated form that might be a precursor for building nucleic acids. So I told Kornberg that what I wanted to do was work on this enzyme-AMP complex.

Hughes: What was his reaction?

Berg: He said it was bunk.

Hughes: Why?

Berg: Because the hypothesis was based on a particular measurement they made, which was to take ATP and incubate it with, quote, "an enzyme," which was a very murky, crude enzyme, and show that the two terminal phosphates would exchange with free radioactive pyrophosphate in the medium. If ATP reacted with the enzyme, you

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[1] Lipmann received the Nobel Prize in 1953.

would liberate inorganic pyrophosphate and produce a complex of the enzyme with adenosine monophosphate (AMP). Since they supposed this was a reversible reaction, one would anticipate that radioactive POP in the medium would react with the enzyme-AMP complex to reform ATP which would be radioactively labelled. And that's what Lippmann and Lynen found.

Kornberg had already studied several reactions where ATP exchanged its two terminal phosphates with radioactively labelled pyrophosphate. But his reaction did not occur by making an enzyme-AMP complex, but by another, more conventional, mechanism. So he said, "I don't believe any of it." I said okay, that may be, but I'm going to take a shot at it anyway. I'm going to purify the enzyme to see if it can produce the enzyme AMP, and to characterize it.

Hughes: Was your enzymology pretty good at this point?

Berg: Yes, by that time, I think I knew how to purify an enzyme. I had been doing it in Copenhagen; that was the thing to do. We had discovered a new reaction, isolated the enzyme, purified it to purity, and characterized that system. So, Kornberg said, "Okay, if you want to do it, go ahead."

Hughes: Did he agree somewhat because you had proven yourself?

Berg: Perhaps. Kornberg had a history of directing the work of people in his lab. He more or less dictated what people did. So, it was unusual that he allowed me to do what I wanted to do. It was not central to what was going on in the lab. But from his estimate of my ability or reputation, he let me do it, even though he predicted it was going to be a waste. And I said, "Well, we'll find out if it's wrong. Because when I purify the enzyme, we're either going to prove that this is true or it's not true." So, as long as I was willing to approach it from the point of view of purifying the enzyme, that was okay.

The reaction I'm referring to was:
ATP + acetate + coA <-> acetyl coA + AMP + PP_i

Lipmann and Lynen proposed that the reaction occurred in three steps:

ATP = Enzyme <-> AMP-enzyme + PP_i
AMP-enzyme + coA <-> Enzyme-coA + AMP
Enzyme-coA + acetate <-> acetyl coA + AMP

The novel feature was the creation of enzyme-AMP and enzyme coA. The evidence for their proposal was that radioactive PP_i was

quickly incorporated into the two terminal phosphates of ATP with the enzyme alone, none of the other substrates.

So, I started off, and very quickly I found that, as I purified the enzyme, there was no longer any exchange of pyrophosphate with ATP, even though the enzyme was capable of catalyzing the overall reaction. I began to ask, "How could Lipmann and Lynen have possibly gotten those results?" These were two very, very distinguished scientists who put their name on a paper that was offering this hypothesis.

And so I began to ask, "Well, what if I tried to carry out this reaction not in totality, but just parts of it?" In other words, I'd leave out one of the substrates so the reaction could not go all the way. And what I discovered is that when I added acetate, one of the other substrates, to the mixture of ATP, pyrophosphate, and the enzyme, it reconstituted the exchange reaction. That indicated that acetate was needed in addition to the enzyme to promote the exchange. Clearly, Lipmann and Lynen were wrong!

My observations indicated that the enzyme catalyzed a reaction between ATP and acetate to form acetyl-AMP.

ATP + acetate <-> acetyl AMP + PP_i
That would make the subsequent reaction:
acetyl-AMP + coA <-> acetyl-coA + AMP

You see that these two steps produce the known products but not by the three steps proposed by Lipmann and Lynen.

Hughes: How do you work out a previously unknown molecule?

Berg: One clue was the existence of a somewhat similar compound called acetyl phosphate--m-acetyl adenylate. The most likely structure was that acetate was linked to the phosphate of AMP. It then required that we prove the existence of acetyl-AMP and show that it behaved in the subsequent reaction as indicated.

So the question is, how do you prove the model? I went to the chemistry department and talked to one of the people there and said, "If I wanted to make something called acetyl AMP, how would I do it?" People knew how to make acetyl phosphate. You just took acetyl chloride and mixed it with silver phosphate, and lo and behold, you got acetyl phosphate. So, I said, "Well, I'm going to take silver adenylate and acetyl chloride, and I'm going to mix them. And I did. And I got an unholy mixture-mess. But out of it I purified something which when I added it to this enzyme, plus pyrophosphate, gave massive amounts of ATP.

Then, working out the rest of the reaction was pretty simple. Kornberg was always there saying, "Wow, great," and so on. And then there was a national meeting, the Federation [for Experimental Biology] meetings, in 1955, I guess it was. I went to that meeting, and the word got around of what I had done. So, I was invited to give a special presentation, which I did. And it blew everybody away, including both Lipmann and Lynen, who were there.

Hughes: What reaction did they have?

Berg: Embarrassment. Sort of a mixture of admiration and embarrassment. Lipmann told me he could never live down, in his own mind, having made such a stupid mistake. As it turned out, they had taken a concentrated extract of yeast, which was like mud, and because they had grown the yeast on acetic acid, it was not surprising that their extract contained some acetic acid, which was all you needed to trigger that reaction.

Lynen, in his more sober moments, told me it was the only time that anybody had shown him to be wrong. And so for me it was kind of a big high, because here I was a postdoc and had come on to solve the mechanism of an important biologic reaction and triumphed over Lipmann and Lynen. This turned out to be a very general reaction, because all fatty acids are activated this way. This was the first step in the process by which you build up long-chain fatty acids.

Rising Star

Berg: So, there I was in a pretty early stage of my career already with two big hits. And in publishing this work--I think there were about three papers--Kornberg did not put his name on them, which I think was a first and last. I don't know of any other paper or work that has come out under his tutelage on which he did not put his name. By his not putting his name on it, I got the full glory. Had his name been on them, there's no question, I would not have profited and benefitted from the discovery in the same way because, as is often the case, the senior author gets a lot of the credit for it. And the people who did the work, who actually had the insight, or who even came up with the idea, get second billing. I have always been extraordinarily grateful to him for doing that.

I don't know whether Kornberg understood that, or whether he just felt he had not suggested that problem to me. I had insisted

on giving it a try. While he was there for me to talk to all the time, and was always available for me to bounce ideas--why I couldn't get this to work or that--he in the end felt it was my work. There was a series of papers. I eventually described the synthesis of this previously unknown compound and worked out the structure.

So by the time I got to this stage, which was 1955, I was pretty well-known in the field. And Kornberg had already asked me to stay on in the department. Even though he didn't have a faculty appointment for me, the school put me up for a Scholar in Cancer Research appointment. It was a special fellowship which was to provide a three-year appointment, with the understanding that the university would give you an academic appointment at the first opening. But you would be in this interim position. It's like the Markey Fellowship, which serves to bridge the period between completing a postdoc and having the first faculty appointment.

Hughes: [Shows Berg his curriculum vitae].

Berg: Yes, "Scholar in Cancer Research, American Cancer Society." It was for 1955, and then in '56, at the first opening, I was appointed assistant professor.

Hughes: So you worked independently at a much earlier stage than is usual.

Berg: Yes, even in graduate school, nobody had assigned a problem to me for my Ph.D. thesis, which is usually the way things work. But I had suggested an idea of how to solve a particular problem, so that was a kind of early independence. Even though you're never really totally independent; you get a lot of feedback from people all the time.

When I went to Copenhagen, I had suggested trying to test Rosenberg's hypothesis in a way that, as many experiments do, leads you into something that was totally unexpected. And then coming to Kornberg with a preconceived notion about what I wanted to work on, which was different from what was going on in the lab and from what he had suggested I might do. And then this work led on to the discovery that a similar kind of reaction happens with amino acids, and that the first reaction in the assembly of proteins is to modify amino acids by their attachment to AMP and then be able to attach them to RNA molecules and allow them to be assembled into proteins. The discovery of this form of activation, as it was called, turned out to have great general applicability and significance. And so, it was seen as sort of a major discovery.

Kornberg's Background

Hughes: Not only was Kornberg an M.D. rather than a Ph.D., but he also had never had formal training in biochemistry. Was that a consideration in terms of your working with him?

Berg: No, not at all.

Hughes: You looked at what he had achieved.

Berg: Oh, yes. First of all, many European scientists, [Severo] Ochoa and Cori, they were all M.D.-trained. In Europe, it was traditional that if you wanted to research in the biological area, you got an M.D. training. With Kornberg, I don't remember that I even thought one second about it, although all the people that I had been affiliated with as a graduate student were always straight Ph.D.s. I don't remember that it ever occurred to me to wonder. In fact, almost all the work Kornberg did was only tangentially related to medicine.

As a medical student, he did research in his own spare time, and the experiments he did were almost always sort of biochemical in nature. He became interested in nutrition. He finished his medical training, and he went into the coast guard, and he was assigned to a ship. He probably told you the experience of trying to take a crew man's tonsil out. They were out at sea, and this guy was in terrible agony with this inflamed tonsil, and Kornberg was the only physician on board. So he went in and excised this guy's tonsil, only he was not anesthetized. That experience taught him that medicine was not what he wanted to do, and so he got transferred to the Commissioned Corps of the NIH [National Institutes of Health], while he was actually in military service. When he left the Public Health Service, he was a commander, or something, in the navy rank.

When he went to the NIH, he started working on nutrition.

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Berg: So, as Arthur says, he realized that there's all this happening within the animal, and you can't tell what's going on. So, he decided enzymes were something that he had to learn about. He went to work with Cori and then with Ochoa. Ochoa was a real enzymologist. And I think Arthur learned a lot of enzymology during those two stays, particularly since there were graduate students and postdocs in the lab, and he mixed with them. When he went back to the NIH, he was finished with nutrition. And I don't know if it was immediately after that he became the head of the

laboratory [Chief of Enzyme and Metabolism Section, National Institute of Arthritis and Metabolic Diseases]. Soon after, there were, in succession, spectacular discoveries of new reactions, purified enzymes, nailing down the mechanism of the reaction--just one after another. That's when [1951] he got this Paul-Lewis Award in Enzyme Chemistry for the enzyme work.

I also recognized myself that enzymes were in the end the only level at which we could understand the biochemistry, so just starting out, we realized we had to get some training and experience with enzymes. Kalckar was doing enzymology, purifying enzymes, measuring them spectrophotometrically. Arthur was purifying enzymes. They were both identified as rising stars. And so an ambitious young guy is going to definitely go to the place where the action is.

Hughes: And that's what the ambitious young guy did. [laughter]

III RESEARCH INTERESTS: NEW AND CONTINUING

[Interview 2: August 12, 1997] ##

Research on Fatty Acid Activation

Returning to a Curious Reaction

Hughes: Dr. Berg, last time we were talking about St. Louis, and you have more to say about the research that you did there.

Berg: The work concerned how acetic acid is activated for its ultimate incorporation into long-chain fatty acids. The mechanism was novel and had not been seen before. Now, in the course of purifying the enzyme that carried out the formation of acetyl CoA, I had come across another reaction, which clearly was not that system, but which had the same characteristics. Namely, it catalyzed the exchange of inorganic pyrophosphate with ATP, but instead of acetate being the promoter of this reaction, it was an amino acid, methionine.

That was curious. It wasn't at all clear why methionine should be involved in promoting a reaction like fatty acid activation. I put that on the shelf and waited until I completely clarified the fatty acid system. And when that was done, I went back to this reaction with the amino acid, and for the life of me, I couldn't figure it out. This was a reaction with no net charge. With only ATP, the amino acid methionine, and radioactive pyrophosphate, the pyrophosphate would exchange with ATP very rapidly. Nothing would happen in the absence of methionine.

Some reaction was occurring between ATP and methionine that was causing the release of pyrophosphate, and that reaction was reversible, so you could reincorporate pyrophosphate back into ATP. Well, I had studied the fatty acid system, which had exactly the same characteristics, namely in that case the enzyme uses

acetate to promote the exchange of ATP and pyrophosphate. Now the acetate system has another component to it, which is coenzyme A, and coenzyme A acts as an acceptor of the activated acetate. The entire reaction involves first the reaction between ATP and acetate to activate, and then to transfer the activated acid to acetyl CoA. So I reckoned that the amino acid system must be very similar, that what we were doing was activating the amino acid to some form, and there had to be an acceptor. But there was no known acceptor. So the question was, why don't we try to find out what the acceptor is? We could easily devise an assay that if you had the acceptor the reaction would go all the way, instead of just going back and forth.

James Ofengand's Research on an Acceptor

Berg: The first graduate student I had, a fellow named James Ofengand, arrived and he was put on that problem. I guess it would have been 1955, because he finished in four years and left when we moved to Stanford. So Jim started to try to identify something which can act as an acceptor of this activated amino acid. Sure enough, he fractionated crude materials and found something which did in fact act as an acceptor. And when he ultimately purified it, it turned out to be a small RNA molecule, which subsequently was called transfer RNA [tRNA]. And so the reaction was completely parallel to the fatty acid system. Only in this case an amino acid was being activated. It turned out that the amino acid was activated through its carboxyl group, just as the fatty acid was. When it's activated through its carboxyl group, the amino acid is very, very efficiently transferred to the tRNA. The interest was that it looked like it might be involved as one of the intermediates in the assembly of proteins.

Paul Zamecnik's Research on Amino Acid Incorporation In Vitro

Berg: There were indications from other laboratories that amino acids had to interact with RNA before being incorporated into proteins. Paul Zamecnik at Harvard and Mahlon Hoagland, who was collaborating with him, had been able to show that amino acids could be incorporated into proteins in vitro, and that a molecule which they called soluble RNA was required. It turned out the soluble RNA was the same as transfer RNA [tRNA]. So, they showed

there was an RNA requirement for incorporating amino acids into proteins.

Purifying Enzymes Activating Some Amino Acids

Berg: We found that the amino acids were being attached to this tRNA, and we reckoned that the amino acid-tRNA was the precursor or the donor of the amino acid for the assembly of protein chains.

Hughes: What year was this?

Berg: This was 1956; it could be '57, somewhere in that period. And I think our first published works on the activation of amino acids is in that period. Subsequently, we went on to show that if you looked back in crude extracts, you found that there were enzymes to activate different amino acids. By the time we came here [1959], we had purified the enzymes that were necessary for activating some six or seven amino acids, each enzyme being specific for a single amino acid, and each being specific for a separate tRNA molecule.

It was our work that really established this notion of one tRNA per amino acid. You could see that there were twenty different enzymes; each one of them could recognize one of the twenty amino acids to the exclusion of the nineteen others, and in the presence of ATP activated that amino acid, and then transferred it to its acceptor tRNA.

Hughes: It sounds as though you were not provoked by Watson and Crick and the central dogma: DNA, to RNA, to protein. You came across this by a strictly biochemical route.

Berg: Oh, yes. The central dogma at that time was, DNA is responsible for encoding proteins. But the "DNA to RNA" wasn't even known. There was a presumption that RNA was involved, but actually there was a misconception. It was thought that the RNA that was involved was in the ribosomes. The ribosomes are the machines on which the proteins are assembled. But in point of fact, it isn't the RNA in the ribosomes which is important; it's the so-called messenger RNA. And the messenger RNA hypothesis wasn't made until 1959. So, we were just following our nose and saying, how do you assemble proteins?

The Zamecnik group was the first one to create an in vitro system that showed that if you put in radioactive amino acids, they could end up in proteins. If you were a biochemist you would

say, "Now, let's fractionate this system and ask what are the important elements for that reaction?"

We came at it quite from the back road. We weren't interested in protein synthesis. We were fresh out of finding the mechanism for activating fatty acids and stumbled, if you will, on the fact that a similar kind of system was necessary for activating amino acids. And once we recognized that enzymes were putting amino acids on tRNA, and knowing of the Zamecnik work, we were in up to our ears in protein synthesis at that point. So then the question to ask is, attaching an amino acid to a tRNA, and each amino acid to a different tRNA, how are all of those amino acetyl tRNAs, as they're called, used in protein synthesis?

Mike Chamberlain and Bill Wood

Berg: When I came here in 1959, I had two superb students. One was Mike Chamberlain, who's a professor at Berkeley, and Bill Wood, who came from Harvard; both of them came as Harvard undergraduates and joined the lab. Bill Wood is a professor at Colorado, in Boulder.

What Bill did was to set up an in vitro protein synthesis system in our lab. He purified ribosomes, and he prepared a fraction which contained all of these enzymes and all of these tRNAs, and demonstrated that the amino acetyl tRNAs were in fact the precursor for incorporating amino acids into protein. Mike Chamberlain was one of the very first people to purify RNA polymerase, because now we knew that there was a messenger RNA. The messenger RNA must have been made off the DNA.

So what was the enzyme responsible for making messenger RNA? Mike Chamberlain, starting with crude extract of *E. coli*, detected an activity of converting ATP, UTP, GTP, and CTP into RNA, and he began to purify that. He was probably one of the first to have actually published a procedure for getting purified RNA polymerase, and he did a lot of characterization.

So, we had in the lab then, after we moved here, the expertise for two parts of the system for gene expression. One was transcription, and the other one was translation. Transcription generates the messenger RNA; translation is the mechanism for converting that messenger RNA sequence into a protein sequence.

Arthur Kornberg's Research on DNA Replication

Research on DNA Synthesis

Hughes: If you include Arthur in that picture, you have his work on the very beginning of this process.

Berg: On DNA replication.

Hughes: Arthur arrived at the DNA synthesis problem circuitously as well.[1] His original interest was not specifically in nucleic acid synthesis, as I understand. And you just told me that you arrived at the problem of protein synthesis by a circular route.

Berg: I think ours was probably more accidental than his. He has written that, whether you take it at face value or not--it differs among different people. He says, "The fact that Watson and Crick identified DNA as the genetic material had no bearing on my choice to work on DNA. I worked on DNA because it was an interesting biochemical problem, and I couldn't have cared less if it was the genetic material."[2] I've wondered whether that was a bit disingenuous, but he holds to that.

But it was very clear: when he was working on nucleotide synthesis, he says that he already had in mind that the long-term goal, if you think the way he does, was how will these nucleotides assemble into nucleic acids? So, in St. Louis, he was working on nucleotide synthesis--how you convert purines and pyrimidines into triphosphates. Already in St. Louis, and I think it must have been something like 1957, '58, he began to ask, "Now, if I can make radioactively labelled nucleoside triphosphates, can I find a system that will actually polymerize them into DNA?

And so, the DNA synthesis story actually starts in St. Louis, and as he has described in his book, it's a really an extraordinary story, because it shows how a person who is determined will persist and believe what most people would not have believed. In his initial experiments, the amount of radioisotope incorporation into DNA he got was so small that it was barely above the radiation background. Many people would have ignored that. He maintained that the fifty counts above

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[1] See Arthur Kornberg's oral history, cited p. 8.

[2] Dr. Kornberg made a similar statement in his oral history, cited p. 8.

background of radioactive material had the characteristics of DNA. He could subject them to various kinds of chemical tests and was convinced that the material he was making in his cell-free system had the characteristics of DNA, so he pursued it. He already was onto DNA replication before we left St. Louis.

Hughes: He could have that confidence because he was so sure of his technique? I mean, the fact that he insisted on working with clean enzymes?

Berg: Well, he didn't have clean enzymes. Those experiments were done in extracts.

Hughes: Then why was he so confident?

Berg: Well, you do good controls, and you subject your findings to the most rigorous tests. By tests, what I mean is you ask: if it's DNA, it should behave like this. If it's not DNA, it can't satisfy all these criteria. So, you get a little bit of a reaction, and you put this fairly intensive scrutiny on it. And if it behaves like DNA, you go ahead. And that's what he did. In fact, when he tried to publish the work, it was turned down. He had one heck of a time getting the work published.

Hughes: Why?

Berg: I'm not sure, actually. My recollection is that it was turned down in large part because calling it DNA, without showing it had genetic properties, was sort of an extrapolation. While he had something that behaved chemically like DNA, DNA is supposed to be the genetic material, and he didn't have a test for the genetic activity of what he had made. So, in fact the paper kept being turned down. Eventually, it was published. But the point I wanted to make was, he was already on the track to try and learn something about DNA synthesis.

Hughes: That's another reason why he would pay attention to a finding that was barely above background.

Berg: Yes, that's right. He reasoned that if thymidine was being incorporated into DNA, it had to be converted to something else before it could be incorporated. He suspected that the true precursor must be thymidine triphosphate. So, he set out to chemically synthesize thymidine triphosphate.

And then, of course, the incorporation went much better. The levels were now much more respectable. He had a good assay. He could begin to purify the enzyme that was incorporating thymidine triphosphate. As soon as he began to do a few

purifications, he showed it required the other three precursors of DNA (d[deoxy]ATP, dCTP and dGTP). In crude extracts, they're usually there in trace amounts. You can't show that they're required, because you're getting a very small amount anyway. But once you purify a little bit, you now find that thymidine triphosphate by itself is not incorporated; you need all the others. That gives you more confidence that what you're making is in fact DNA.

Hughes: Yes, I see.

Berg: So, it's kind of the dogged biochemical approach, and I think the faith--and it is faith--that you can produce in vitro what must happen in the cell, and that you have to purify it to eventually be able to uncover the mechanism.

Nearest Neighbor Experiment

Berg: Actually, before we left St. Louis, Arthur had already established the parameters of that DNA system, to which many people refer even today. He was able to show, in what he called the nearest neighbor experiment, that the frequency with which a nucleotide goes in next to another nulceotide is exactly the same as [the frequency] at which it occurs in the DNA used as a template. And he proved some important points about DNA replication: that it had to be, what we called, anti-parallel synthesis, which was not proven by the Watson and Crick structure.

DNA as the Genetic Material

Hughes: In the paper that he had trouble publishing, did he show that the four bases were necessary?

Berg: I think so.

Hughes: And that still was too much of a leap of faith to say, this is DNA; this is the genetic material.

Berg: Right. I don't remember that the issue was whether somebody would believe that DNA was the genetic material. In fact, it was the other way around. It almost was that the true nonbelievers were the people who said, "Well, DNA has a property; it's the genetic material; it confers genetic specificity. How do we know that

what you've made is DNA? It was actually 1967 before he actually showed that he could make a viral genome.

Despite his statement that he didn't care if [DNA] was the genetic material or not, he clearly tried at various stages to determine whether the material they were making did have some genetic property. They tried to make transforming principle, but that didn't work. He accepted that DNA was the genetic material, and he was now making it in a test tube. The ultimate thing would have been to prove that what he made in the test tube had biological activity. Although, in the beginning I'm quite sure that wasn't the motivation.

So, Arthur's getting into DNA replication was less accidental than our getting into protein synthesis, which was purely a result of the fact that we had stumbled onto a reaction which resembled one which we had been working on. What got us into protein synthesis was that the reaction we found looked like it was producing the precursor to protein synthesis. [interruption]

More on Amino Acid Assembly and Messenger RNA Research

Berg: We worked on that for quite some time. Being the biochemist that I was trained to be, and under Kornberg's tutelage and encouragement, I was pretty much a classical biochemist in his mold: you find a reaction, you purify the system, and you work out the mechanism using pure enzymes. So what happened for probably five or six years after we moved here was to work out this whole system of how amino acids become activated for protein synthesis and the mechanism by which the amino acids actually become assembled into a polypeptide chain. And, in addition, how you make the messenger RNA that actually directs the order of assembly. So those were really the major activities going on, purifying all of these so-called amino acyl tRNA synthetases. As I say, there are twenty different ones.

You might have thought the simplest scheme would be twenty different tRNA's, each one accepting only one amino acid. As it turned out, there are probably closer to a hundred different tRNA's, and for any one amino acid there are probably two, three, or four different tRNA's. We came to understand why that was necessary. The genetic code has sixty-four triplets, sixty-one of which actually specify amino acids. That means that the code is degenerate, meaning there are more codons--triplets--for each

amino acid than just one-to-one. And if you have different triplets than you have to have different tRNA's.

What we studied for some time was, how does the amino acid activating enzyme recognize one amino acid to the exclusion of the nineteen others? Because all the amino acids have a common core; they have a carboxyl group and they have an amino group, and all the amino acids look the same. The only thing that's different is that they carry side chains which are of different kinds. And that means that each enzyme has to distinguish each amino acid on the basis of its side chain.

Some of the amino acids are very similar. It seemed reasonable to ask how could one enzyme distinguish between the amino acids isoleucine and valine, which differ ever so slightly. You would imagine that if an enzyme could recognize isoleucine, it must also be able to recognize valine. We discovered that in fact the enzyme does just that; it makes mistakes. But it has an extraordinary capacity to rectify its mistakes, because once it transfers a wrong amino acid onto a tRNA, it knows it has made a mistake, and it clips it off. So, you never get the wrong amino acid going into a particular position on a protein. We worked out a lot of the details of that specificity.

The second part of the specificity: once the enzyme has made a particular amino acyl AMP [adenosine monophosphate], which is the intermediate, how does it recognize the correct set of tRNAs to the exclusion of the hundred others? All the tRNA's look very much alike. They're all small; they have between seventy and ninety nucleotides; they're all folded in essentially the same way. And yet the protein seems to be able to recognize some small subset, puts the amino acid on those, and doesn't ever put it on the wrong ones. So, we worked out a lot of the basis for that specificity, new assays and so on and so forth.

Hughes: Is it a somewhat unique situation in biochemistry that enzymes are forced to be so discriminating?

Berg: This is probably one of the most discriminating of all. You could say polymerase is almost the other extreme: it uses any one of the four triphosphates. And they differ. I mean, ATP, CTP, and UTP all look very different, yet the enzyme knows how to use each one of them, but dictates which goes in first or next; the order is determined by the template. The enzyme is just there to match whatever it picks up. If it doesn't match, it's released. At random it picks the triphosphates; if they form a hydrogen bond, it will make the linkage.

But in the case of amino acids, activation is very highly specific. Once the amino acid is attached to the tRNA, its fate is sealed. It will go into a position in the protein dictated by the tRNA, not the amino acid. The amino acid could be anything. Seymour Benzer demonstrated in a brilliant experiment that if you put an amino acid on a particular tRNA, and then chemically change the amino acid (cysteine into serine), it goes into the protein in position calling for cysteine, not serine.

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Berg: Thus producing the correct sequence in the protein is determined by the specificity of these activating enzymes. If the activating enzymes make mistakes, then the protein will be incorrect. That's why we focused so much on trying to understand the nature of the specificity and the error-correcting features that the system has. So between 1959 and roughly 1965-'66, we focused on understanding both transcription--the copying of DNA into RNA--and the assembly of amino acids into proteins, specifically on this activation step.

Gene Regulation

Pasteur Institute Contributions

Berg: Meanwhile, of course, a lot was happening working out the holy trinity, which is DNA to RNA to protein. The Paris group at Pasteur pioneered in the field of gene regulation. How do you regulate whether a gene is transcribed or not?

It was known for quite a long time that in normally growing *E. coli*, for example, most of its genes were not working. They're only expressed when the cell needs them. The induced enzymes are enzymes which are not expressed at all unless the cell senses a need to make those enzymes in order to metabolize some material in the medium. Scientists at Pasteur worked out a whole lot of the details of this control mechanism, involving so-called operators and repressors and so on.

Hughes: You had people in your group in St. Louis who had been at the Pasteur Institute.

Berg: Melvin Cohn, David Hogness.

Hughes: Yes, and Dale Kaiser?

Berg: Dale Kaiser worked on bacteriophage. He had been to Pasteur, and he worked with [Andre] Lwoff on a particular aspect of bacteriophage lambda.

Stanford Biochemistry Contributions

Hughes: The idea of gene regulation was already in the wind before you arrived in St. Louis?

Berg: Dave Hogness and Mel Cohn had worked on that in the late fifties and had done one of the classic experiments, which showed that enzyme induction actually involved totally new protein synthesis rather than activation of an inactive form of the bacterial enzyme, and induction is nothing more than conversion of an inactive form to an active form. They showed that when the inducer is present, the cell begins to make proteins de novo, i.e. from scratch. And that proved that induction was really control of protein assembly.

Using genetic tools primarily, [Jacques] Monod and [Francois] Jacob showed that there must be a region upstream of the beta-galactosidase gene which regulates that gene's expression and that there was a repressor that binds to that regulatory region blocking gene expression. Thus, when the repressor is bound to that region of the DNA, beta-galactosidase is not made. In this case, the inducer binds to the repressor causing it to come off the DNA, allowing expression of the gene to go on. The details were worked out I'd say in the late fifties to early sixties when Monod and Jacob and their colleagues did their famous work identifying the operator (the control region) and the operon (groups of genes that are regulated together).

We were following that work closely because Mel Cohn who had worked with Monod was in our department, and he was educating us. Most of us were biochemists; we were not geneticists, and we were not microbiologists, but Mel Cohn was both. And so we were learning about what was going on in Pasteur through him. In fact, Monod and Jacob came many times to St. Louis and we got to know them. We became friends with them as well as discussing their research with them.

Dale Kaiser and Bacteriophage Lambda

Berg: My best recollection is that sometime in 1965 Dale Kaiser gave a graduate course on bacteriophage lambda. He was really already one of the experts in this field. And he was a wonderful lecturer. The last lecture of his course attempted to draw the analogy between bacteriophage lambda and a mammalian tumor virus.

Now bacteriophage lambda, just to give you a little background, is a virus which has two possible outcomes when it infects *E. coli*. One is, the virus multiplies and the cell dies. It pops open and releases several hundred bacteriophage particles. They go on to infect other cells, those cells lyse, and you produce what's called the lytic phase of bacteriophage lambda infections. That's the way most bacteriophages work. They kill the cells in which they multiply.

But what had been discovered in the Pasteur Institute by Andre Lwoff, with whom Dale Kaiser had worked, was that bacteriophage lambda frequently enters the chromosome of the bacterium and remains dormant. The bacterium continues to multiply perfectly normally, but it has acquired a new set of genetic information, that carried by the bacteriophage genome. But a complicated system keeps most of the genes of the bacteriophage repressed, and so the bacteriophage genome is maintained in the bacterial genome. But with a very, very low frequency, the virus genes are spontaneously activated and the virus begins to multiply and kill the cells. The phenomenon is called lysogeny.

Dale Kaiser was one of the experts of this phenomenon, and that's what he had studied. Cells that are lysogenic cannot be infected, because they're producing a repressor which is keeping all viral genes repressed. So when a new virus chromosome comes in, the repressor blocks the action of its genes, and new infections don't work. Dale had studied this phenomenon called immunity. He was a terrific colleague. He was bright. He brought a whole new world to most of us.

Research on Tumor Viruses

Turning to Tumor Viruses as an Experimental System

Berg: Now, it was already known that there is a system of mammalian viruses which infect cells, integrate their chromosomes into the chromosomes of the cell, and the cell doesn't die. In fact, the cell becomes transformed into a tumor cell. That's why these viruses are called tumor viruses. They rarely cause lysis; they rarely kill the cell.

SV40 and polyoma are two viruses that are characteristic of this group. They are small DNA viruses. When they infect cells, depending upon the type of cells, they either kill the cells and produce more virus particles, or they integrate into the chromosome and remain dormant. In the latter case, they confer a new property on that cell, namely it becomes a tumor cell. When Dale contrasted these two systems, lysogeny with bacteriophage and oncogenesis or tumorogenesis by tumor viruses, he pointed out the striking similarities.

Hughes: That was a novel comparison at the time?

Berg: No, I think people who had been working with the tumor viruses already were aware of lysogeny and were thinking that that might be a compatible system. But it turned out not to be the same because in the bacteriophage a repressor is produced which blocks gene expression in the viral genome. In the case of the mammalian cells, there is no repressor; the virus DNA integrates into the chromosome and becomes stably associated with the host chromosomes. The virus genome can't replicate itself. But it continues to express some of its genes, one or more of which is necessary to convert the infected cell into a tumor cell. So, these tumor viruses carry a gene which causes cancer. As long as a cell retains that virus gene, it grows in an aberrant way. I was intrigued by that. I was getting a little bit tired of working with bacteria.

I should step back for a moment. The studies with the mammalian viruses are done in tissue culture. Just as in the bacterial system you grow *E. coli* on a petri plate and you infect them with a virus, you grow mammalian cells on a petri plate and you infect them with a virus, then you can identify cells that become transformed. I was also well aware that we had learned an enormous amount about molecular biology, gene function, regulation, etcetera, by studying the viruses that infect bacteria.

There were two approaches to exploring molecular biology. One was to study the bacterial cell, and the other one was to study what happens after you infect the bacterial cell with a virus. If you think about it, when you infect the cells a new set of genetic information is brought into the cells. Now you have almost a synchronized system; all the cells are infected, and now the virus's genetic program takes over. So if you want to study gene regulation, you could study the regulation of the bacteriophage's genes, rather than the more complicated ones of the cell. The viruses have a smaller genome, so there are fewer genes to have to worry about. And you have this possibility that you can isolate the genes of the virus because you can grow the virus in large quantities. It's a simpler system, smaller genomes. So a lot was learned using that paradigm.

The lesson I think that I and a lot of people drew was using viruses as a probe or as a model often provided a lot of information about the nature of genetic control and the processes involved in going from gene to protein. So, hearing that there was such a thing as tumor viruses and that they induced cancer in animals was intriguing. But if you look at it, you would say why couldn't you use these viruses in the same way, to study gene regulation in mammalian cells, rather than just in bacteria? I used to joke when I said that what I wanted to know was whether the Jacob-Monod model for regulating gene function was as true in mammalian cells as it was in bacteria.

Hughes: You didn't find the complexity of the mammalian cell daunting?

Berg: Well, I didn't, because I was not going to be studying the mammalian cell genome's expression and regulation. I was going to be looking at the expression of the viral genes after the virus infects.

Hughes: I see.

Simian Virus 40

Berg: The viruses that we chose to work on had only five genes. They have a very small DNA genome; a circular DNA molecule of only about five thousand base pairs.

Hughes: Both SV40 and polyoma?

Berg: Yes. SV40 grows in human cells and primate cells; polyoma grows in mouse cells.

Hughes: And their simplicity is at least partially the reason they are research tools?

Berg: I think the simplicity really only became apparent in the mid-sixties. SV40 was discovered in 1961, inadvertently. It turned out that SV40 was a contaminant in the Jonas Salk polio vaccine. The Salk vaccine was prepared from polio viruses that had been grown on Rhesus monkey kidney cells. Virus was recovered in the cell lysates without much purification and then inactivated with an agent that kills the virus but doesn't destroy its ability to act as a vaccine. It turned out that these rhesus monkey kidney cells were infected with SV40, which nobody had ever known. SV40 stands for simian virus. It was discovered by taking polio virus and injecting it into hamsters; much to people's dismay, the hamsters developed tumors. That was very worrisome because the same vaccine was being injected into kids. Therein lies one of the things that we'll come to in the recombinant DNA controversy, because we were using SV40, and the question was, does SV40 produce tumors in humans? It certainly produces tumors in rodents. The question was, is working with SV40 dangerous?

Kornberg's Dedication to *E. coli* as a Research Tool

Berg: After hearing Dale Kaiser's lecture, I decided that what I wanted to do was to stop working on bacterial systems and learn how to culture mammalian cells and to use SV40 or polyoma as a model for studying gene expression and regulation of mammalian cells.

Hughes: Were your colleagues behind this?

Berg: Arthur was furious. I won't say we ever came to blows, but there were times when I was so furious with him because he was so critical. He more or less said, "You're wasting your talent. You're destroying your career. You have so much of a gift for doing enzyme research. The only true path to knowledge is *E. coli*," and so on and so forth. He was very narrow-minded. I won't say he forbad me from taking that step, but he certainly predicted complete failure. The fact that we didn't fail and that we turned up a lot of very important new things about SV40, which I'll come to, made him even less happy. In fact, the term he used is that I was a Pied Piper leading people astray, taking them away from important basic research into this messy field of working with uncharacterized systems, complex systems like mammalian cells and so on.

Arthur and I have this debate to this day. For example, if you want to study immunity, you can only study immunity in a system in which immunity exists. You can't study immunity in *E. coli*. But he won't concede that. He will argue that principles and models that you learned from *E. coli* may be the key to unlock the understanding of immunity. For sure, but at some point you have to work with cells that display immunity. If you want to study oncogenesis and tumor formation, you want to learn what the genes are that are responsible for forming tumors. You're not going to find out about tumor genes in *E. coli*. So, that's an ongoing debate. We have it all the time.

And we'll probably have it tonight, because we're having dinner with Senator Connie Mack, who is trying to increase funding for the NIH. I'm sure that tonight at dinner Mike Bishop, Kornberg, and I will get into a debate about which is the true path to knowledge. [laughter] Kornberg will lament the fact that people like me and Bishop have opened up vistas that have been extremely informative and profitable in terms of research and left *E. coli* behind. He talks about fashions in research, and the fashion now is not to work on *E. coli*. The work which went on over the last thirty, forty years, will not be used to its fullest because people are now going off into other research areas.

In fact, if I would tell you some stories that are really amusing: there was a time when I heard Arthur lecture Gobind Khorana for leaving the work that he was doing on the chemistry of nucleotides and nucleic acids to work on membranes, which Arthur thought was a waste of his talent. This was after Gobind had the Nobel Prize. I won't say he berated--that's probably too strong of a word--but he certainly was furious with Francis Crick for seducing his son Roger [Kornberg] to work on chromatin when Roger went to Cambridge to the MRC [Medical Research Council]. "Chromatin was dirty. It was a mess. It was too complex." Yet Roger made some of the major discoveries that opened up the whole field of chromatin to a more sophisticated kind of study.

When I was chairman of the department [1969-1974], I had a big fight with him about a professor in our department who also decided to work on tumor viruses and went off and spent a year in London at the ICRF. George Stark came back and began to leave what he was doing, which was protein chemistry, to work on tumor viruses. Kornberg read him the riot act at one of our retreats. He just told him he was wasting his career, wasting his talent. I was furious, and I told Arthur he had no right to be telling people what to work on.

Anyway, this is a long story, and an ongoing debate that we've had over many years, and continues. We differ over the

intensity of his feeling that working on *E. coli* is the only worthwhile system and everything else is too messy. But in fact, the science of mammalian cells has really blossomed.

Hughes: I understand that Arthur's approach was a relatively common one, probably up until the late fifties, wouldn't you think?

Berg: Yes.

Hughes: The answers were to be obtained by looking at simple systems, and the mammalian cell was too complex an entity and science didn't yet have the tools to approach it.

Choosing a New Research Direction

Berg: What Arthur didn't understand, and what I tried to convey to him on many occasions, was that ambitious, bright, young people want to move away from where everybody else is working. They try to open new fields. If they ask a question that takes them in a totally new direction, that's exciting. They want that challenge.

Arthur was that way. He was a nutritionist. He was working on feeding mice or rats, and he said he got fed up with just studying what went in and what came out and not knowing what went on inside. So, he left nutrition. Now, at that time I'm sure somebody must have said, "You're going to damage your career, because you are one of the leading people in this field. You're going off to work on purified enzymes and take a year off to go work with Ochoa and Cori?" Well, when he came back, he was the hot, young guy in a new field.

I saw my position pretty much in the same way. The bacterial field, particularly in the area of gene expression, in outlines were already becoming quite clear. The Jacob-Monod-Lwoff story was already the dominant paradigm. And yet there was nothing known about gene expression in mammalian cells, in eukaryotes--nothing! It wasn't a totally unnatural question to ask: Are the mechanisms that work in bacteria also the ones that will explain gene regulation in eukaryotic cells? Very few people were working on that.

I was ambitious, reasonably bright, and had already made a mark in traditional biochemistry. I was really interested in trying to do something new, something different, that other people were not doing. And I have no doubt, had Arthur been in my position, he would have done exactly the same thing. I'm

absolutely certain. I think that attitude has driven a number of other people in our department and made the department so successful. Dale Kaiser went into working on a totally new system in developmental biology. Dave Hogness who had been working on bacteriophage X went off and started working on *Drosophila* genetics. Each of them have become the leaders in the new fields that they literally created. Some people can stay with what they did as graduate students, postdocs, and follow along, and some are very successful. But for me, I would get bored.

Sabbatical at the Salk Institute, 1967-1968

Berg: When I chose to work on tumor viruses, it was just a budding field, and one of the leading people in it was Renato Dulbecco. Renato came up to visit Stanford, and I asked him about the chances of working at the Salk Institute. This was because of listening to Dale Kaiser.

So, the question was, do tumor viruses express some genes that cause the cell to behave like a tumor cell? I decided that I was going to take the year off, learn how to grow mammalian cells, and come back to Stanford and work on tumor viruses. That plan did not meet with Arthur's approval; everybody else thought that was great.

My assistant Marianne Dieckmann went with me to La Jolla. I was due to get a postdoctoral fellow who was coming from the Pasteur Institute to work with me. When I changed my plans to go to the Salk Institute, he came there with me. So, we went down there as a small team--myself, Marianne, and Francois Cuzin, who had come from Francois Jacob's lab at the Pasteur Institute. We started working with tumor viruses, adapting the strategies from molecular biology. All the concepts that we had of regulation, of the way bacteriophage multiply, how they lysogenize, we brought with us to the animal virus field. When we came back to Stanford, I was committed; that was the new direction we were going to go in. And the work that was ongoing with the amino acid activating enzymes sort of tailed off.

Establishing Research on SV40 at Stanford

Berg: I was away during '67-'68, and when I came back I built a new laboratory, which had all the safety features--filtered air,

negative pressure, laminar flow hoods, and everything of that sort. The point is I was committed; I was going to work in that field. We did some important work of opening up a whole field: finding mutations, characterizing the genome of SV40--it's a small circular DNA molecule, 5243 gene pairs--and trying to determine which regions of the DNA specified which gene. We worked out a whole lot of new technology for doing that. It was built on methods that we brought with us from the earlier work with bacteriophages, which is usually the way science progresses.

Building on the Bacteriophage Lambda-*E. coli* Research

Hughes: Your concept was that what happens in *E. coli* infection is a basis for thinking about what happens in mammalian cells?

Berg: Absolutely. For example, the virus chromosome is a small DNA molecule; it has to be transcribed; the cell's transcription machinery resembles that of bacteria in having an RNA polymerase which it uses the triphosphates, and so on. So what you know about transcription in *E. coli*, you're going to immediately apply to transcription in eukaryotes. It was possible to map the RNAs and to characterize them, that is, when they're made after the infection. As it turns out, when the virus enters the cell, part of its genome is transcribed into what we call early messenger RNAs. Those early messengers make early proteins.

Those early proteins then start the system of replication, and you then begin to express a new set of genes, so-called late genes. The late genes encode the shell of the virus, very similar to that of the bacteriophage. Bacteriophage lambda T-4 do almost the same thing: they enter *E. coli* and express part of their genome, early genes; they make early proteins; early proteins start replication. The replication initiates late transcription which results in the proteins that form the phage. Very similar, right? But now you've opened up a whole new world of how a mammalian cell does that. And you can begin to study the mechanism by which mammalian cells carry out the basic steps of gene expression.

Drawbacks of Using Commercial Reagents

Hughes: What difference does it make that students today come to mammalian cell research without the experience that you and others of your

generation had? Do they lose something because of not having experience with phage and *E. coli*?

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Berg: I think the big difference today is students are unaware of the amount of work it takes to prepare all of their own reagents. When Kornberg was studying DNA replication, he had to synthesize all the radioactive deoxytriphosphates. To the people in his lab, it was like being sentenced to Siberia when it was their turn to take several months out and prepare the labeled triphosphates. Today, students just write an order and buy it. They buy all their enzymes; they don't purify any enzymes--ever. And when they buy them, they have no respect for their use. They never worry about how much activity there is; they just dump in some amount. The manufacturer says use five microliters; they don't know what five microliters contain. They don't know if the enzymes have impurities and so on and so forth.

Those of us who grew up in a more traditional mode worry about all these things. If you get a result that's funny, maybe it's funny because your enzyme preparation is contaminated, not because there's some funny phenomenon. But people who have moved into the animal field, who work with *Drosophila*, are very sophisticated.

Biochemical and Genetic Approaches

Berg: Paramount today is the genetic approach. In working on the Beadle biography,[1] I've come to recognize the power of the approach which he exploited, which in fact even preceded him. If you have a complex biological phenomenon or system, you can approach it in two ways. Kornberg's approach is to break open the cell and try to identify some reaction you're interested in, and purify the components responsible for the reaction. The other approach is to make mutants--ones which block the phenomenon, obliterate the phenomenon, change the phenomenon, or prevent the reactions that you're interested in studying from happening.

The various steps that are involved in this process are dissected by making mutations in each and every one of the steps. Consider a complex pathway in which a very simple precursor is

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[1] At the time of the interviews, Dr. Berg and Dr. Maxine Singer were collaborating on a biography of George Beadle.

converted via ten steps to an end product, and each step is catalyzed by an enzyme. You could try to purify all of those ten enzymes and work out the detailed chemistry of each step. Or you could start by making mutations which identify the steps, because when you make a mutation that blocks a step in the process the substrate of that step accumulates. Because they accumulate, the intermediates in the reaction can be identified.

Having mutants provides a powerful tool to look for the protein or the genes which encode the protein. If you have cells that can't carry out one of the steps in the pathway, you can now use such cells to isolate the gene, because introducing that gene into these cells overcomes the block. These are the two different paradigms for trying to analyze complex biological systems.

Mutations are the predominant approach when we have no idea what reactions are involved in the complex system under study. Fly behavior is a good example, for example, phototropism. Normal flies move to a light source, and it is possible to obtain mutants that fail to do that. You get mutants that can't see, mutants that can't fly, and mutants that can't translate the light signal.

This [genetic] paradigm is what people are doing today. It's no less honorable than purifying enzymes. Some enzymologists put their faith in enzymology, but I suppose I'm more liberal. I admire, accept and encourage people who want to try new ways to do things and don't insisting that purifying enzymes is the only way.

Fluid Disciplinary Boundaries and Multidisciplinarity

Hughes: Is it difficult to label what people in the biochemistry department actually are? Are they biochemists, geneticists, or cell biologists?

Berg: I think that's happening in all areas. In the Pharmacology Department, you couldn't distinguish two-thirds of the people over there from the people in my department. Similarly, in Microbiology, people are doing the same kinds of things that are being done in Biochemistry. The artificial definitions don't prevail anymore. Many scientific problems have now gotten to be very complex and really need multiple and different kinds of approaches for their solutions.

One of my jobs here at the Beckman Center is to create mechanisms that bring people together from different departments, ones who bring different perspectives, different skills, different

technologies to a problem. The ability to solve complicated problems rarely exists in any one department any longer. The problems are too complicated. So, what we're trying to do is to create interdepartmental programs around themes, around areas of interest.

Kornberg tends to resist these new approaches. Arthur remains a strong advocate for the enzymological approach, using pure enzymes. He can admire new approaches, but he'll still say, "You don't really understand it until you get the enzymes out and purify them." That's good. It serves a very important function. You can make the argument that the purpose of the mutational approach is to identify the genes responsible for the individual steps. Ultimately, however, to understand that pathway, you must purify, identify, and characterize the proteins that perform those steps. Nobody denies that.

One time when we were recruiting a new faculty member, Arthur and I interviewed a young woman who had already done some really fantastic work at the MRC [Medical Research Council] in Cambridge, using the nematode as a model for studying development. We brought her over. She gave a brilliant seminar. Kornberg asked her why she wanted to be in a biochemistry department if she was a developmental biologist. She explained that the approach she was using, which was to obtain mutants, was only to get into the system and that ultimately she wanted to be able to understand these mutations at the biochemical level. Then Arthur asked her, "Why should the biochemistry department be interested in you, a developmental biologist?" And she said, "I didn't ask to come; you invited me to come here." [laughter] She was pretty good. She's at UCSF now and one of their real stars.

Hughes: Who is that?

Berg: Her name is Cynthia Kenyon. She's now one of the leaders in the nematode field and using nematodes to study aging. She's been making mutants whose life spans are much longer than normal. Interestingly enough, Arthur's son Tom, at UCSF, is using *Drosophilia* mutants to study their embryonic development. So there you are.

Research Leading to Recombinant DNA Work

Hughes: Let's discuss your recombinant DNA work.

Berg: A reasonable starting point is to ask how did we ever get to thinking about recombinant DNA while working on SV40?

In moving from one field to another, you bring to it some background, some insight and some prejudices, if you will. As I was preparing to go off on sabbatical, I was getting more and more involved with using genetic tools to study how amino acyl-tRNAs work in protein synthesis.

Collaborating with Charles Yanofsky on Suppressive Mutations

Berg: One of my colleagues in the biology department, Charles Yanofsky, and I became very close friends, and we began to collaborate on certain experiments. He had demonstrated that certain kinds of mutations could reverse the effects of other mutations; some mutational suppressors appeared to work through mutations in tRNAs. That is, if a tRNA is supposed to read a codon A-B-C, you can get a mutation in the gene that specifies this tRNA so it now reads A-B-D, and not A-B-C. As a consequence, a particular amino acid will be inserted into the protein chain in the wrong place. Such mistakes can reverse the effects of a mutation in the protein coding gene; hence the term suppressor.

Using Phage as Transducing Agents

Berg: We were studying mutations which affect tRNAs that change the reading or the translation of the genetic code. In order to do that, I was beginning to learn to do a lot of genetic crosses with bacteria and using bacteriophage for what we call transducing agents. Lambda is one of a whole series of phages which, after integrating into the chromosome and then coming out, incorporate pieces of chromosome into their own chromosomes.

Hughes: By mistake?

Berg: More or less by mistake. So, they integrate at a particular place, and with some low frequency, they come out, but incorrectly, picking up a piece of bacterial DNA while losing a bit of their own. When such virus particles infect another cell, what do they do? They bring with them the information that they stole from the first cell. So, you can transfer genes from one cell to another by bacteriophages. And that proved to be a very

powerful technique in altering the genetics of cells by bringing in genes from different sources.

Hughes: Could you specify the gene to be carried?

Berg: Yes, because some bacteriophages, like lambda and another one called phi80, integrate in only one place in the bacterial genome. So when they come out, they can only bring out the pieces that are alongside. There are other phages, however, that cause the bacterial genome to be fragmented into small pieces, and when the phage is assembled, it picks up cellular DNA pieces at random. The pieces it picks up are roughly the size of its normal genome, and the particle looks like a bacteriophage, but it has a piece of bacterial DNA instead of phage DNA. The pieces are picked up at random. Consequently, some phages are carrying one gene, another phage is carrying a different gene, another phage is carrying a different gene, and so on.

This notion that viruses could carry genes from one cell to another really was a reality, and I was using it all the time. It's called transduction. So phage could transduce cells with a variety of genes from any cells in which the phage was grown.

Developing a Transducing System for Mammalian Cells

Berg: Well, while I was working on the tumor viruses, the question I asked myself was, are mammalian viruses capable of picking up mammalian viruses and bringing them into new mammalian cells? In other words, could you, in fact, develop a transduction system that works for mammalian cells, just as the bacteriophage work with bacterial cells? The reason for believing that was possible is that when I was at the Salk Institute, phage particles that carried cellular DNA were discovered. The question was, could those viruses that are carrying cellular DNAs be used to transform other cells? After making some calculations, it became quite clear that SV40 and polyoma couldn't do it, because their genomes were very small. Their genomes comprise only five thousand base pairs, and you can't pack more into the virus particle. So, the most you could possibly pick up from a mammalian cell would be five thousand base pairs.

When bacteriophage picks up cellular genes, it loses a little bit of its own. But lambda is fifty thousand base pairs; SV40 is only five thousand. So, the question is, how much could you lose, and how much could you actually pack in? The answer was that the amount you could pack in to the virus particle is hardly

big enough for a single gene. And even if it was big enough for a single gene, the likelihood that any particle contains the gene you're interested in or knew how to look for was problematic, like looking for the needle in a very large haystack.

I knew that we had to find a way to move genes from one cell to another. We thought that if we couldn't find virus particles that had picked up a specific cellular DNA, then why not make them? We wondered if we could we take a set of pure genes and insert them into the SV40 genome in vitro. If we could, then we might use the virus DNA's ability to enter a mammalian cell, integrate into the chromosome, and carry with it whatever had been attached to it.

Hughes: The reason that you were interested in the DNA of SV40 was simply as a transporting mechanism?

Berg: Absolutely. That was the notion we had. If we could create such a system, it would really greatly enlarge the capability of studying mammalian cells and doing molecular and cell biology. A question was, what could we attach to SV40?

At that time in Dale Kaiser's lab, Ken-ichi Matsubara was working with a small piece of bacterial DNA that could replicate in *E. coli*. It was part lambda bacteriophage DNA associated with three genes from *E. coli*. This plasmid could be purified and obtained in pure form. The plasmid, called lambda dv gal, was about five thousand base pairs in length and contained three bacterial genes and the little piece of lambda DNA which allowed it to replicate in *E. coli*. We had pure SV40 DNA, and we knew that SV40 DNA could be used to infect mammalian cells, whereupon new viruses are produced, or the DNA integrates into the cells' chromosomes.

We had pure SV40 DNA which contains about five thousand base pairs in the form of a ring. We thought that if we could make a molecule which contains SV40 DNA as well as the bacterial genes, we could ask if, after introduction into a mammalian cell, the bacterial genes are expressed. That was the question we set out to answer.

Lambda Bacteriophage with Complementary Tails

Berg: First we had to figure out how do you join two DNA molecules. A colleague at Stanford, Dale Kaiser, had been studying a lambda bacteriophage's cohesive or sticky ends. The phage DNA has

single-strand protrusions from each end which allow molecules to join end-to-end. Under certain conditions, lambda DNA could join end to end to make long chains or under other conditions they circularize. So the existence and behavior of cohesive ends in a lambda phage DNA pointed the way to do the joining. If you make complementary tails on DNAs, those DNA will join to one another through the formation of hydrogen bands.

Hughes: You mean literally make the tails, synthesize them?

Berg: Phage lambda DNA has them naturally. What Dale Kaiser and Al Hershey and a few other people all discovered simultaneously was that these ends are like Velcro. They're cohesive, because they're complementary to each other. And so under the appropriate conditions, a linear molecule of lambda will form circles, or [join] end to end to produce long chains.

Synthesizing "Sticky Ends"

Berg: So we set about to learn how to synthesize synthetic sticky ends, because the two DNA molecules we had did not have cohesive ends. Once you were aware of the fact that cohesive ends exist naturally and allow DNA molecules to join one to another, you would say the trick is to make synthetic ends. Because strands of A's and one with T's pair, it seemed logical to put tails of A on one [DNA molecule] and tails of T on the other, and after mixing them, they very likely would join. So, the task became one of creating sticky ends with an enzyme.

DNA Ligase

Berg: Now most of the enzymes for doing this whole operation were in Kornberg's refrigerator, and we had access to all of them, which was one of the great things about the department.

Hughes: There were several people involved with isolating ligase, not all of them at Stanford.

Berg: Three labs get credit for discovering the enzyme DNA ligase virtually simultaneously. Bob Lehman, Kornberg, and Martin Gellert.

Hughes: If you've got base pairing, where does the ligase come in?

Berg: Normally, SV40 DNA is a closed circle, that is, there are no nicks or gaps; each chain is absolutely continuous. If you circularize a linear molecule as I described it, a DNA ligase is needed to join all the ends. In the case of lambda DNA, when you circularize it, there's perfect fit right up to where you make the ligase join the ends creating the closed circles. With uneven tails of A's and T's, circularization occurs, but these are gaps. These have to be filled in before ligase can join the ends.

Recombinant DNA

Making Recombinants of SV40 and Lambda dv gal

Berg: Making synthetic tails was the first step [sketching] [See diagram A, page 68a]. Let's see, these are A's, and these lengths are variable because we had no way of controlling their lengths. With dATP and an enzyme (deoxynucleotidyl transferase) A's are polymerized onto the ends. We try to put on somewhere in the range of a hundred to a hundred and fifty A's per end. But the two tails are certainly not the same length. Now T's are added with dTTP and the same enzyme. When the two DNAs are joined, there are four gaps because the dA and dT tails are not all the same length.

Hughes: I see that.

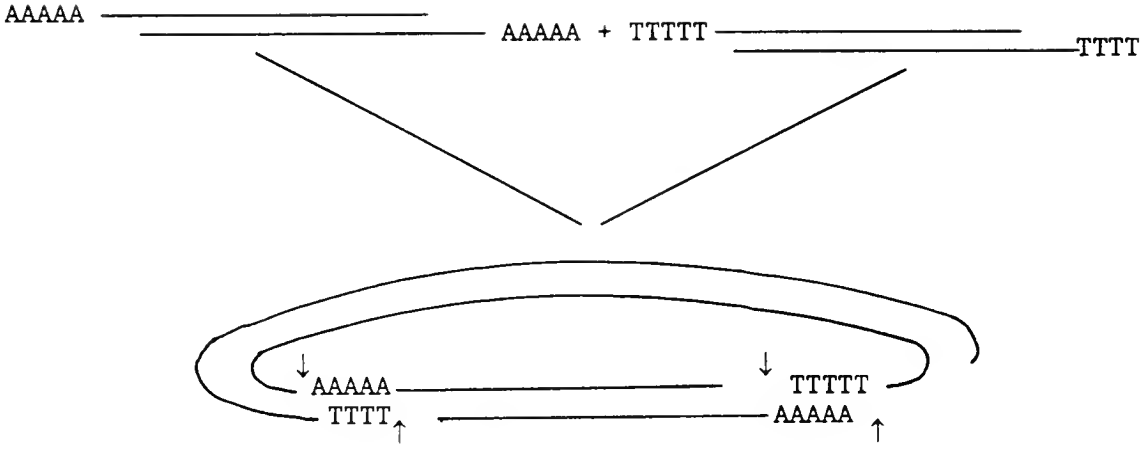
Berg: To fill in the gaps and seal the ends we used DNA polymerase I, the Kornberg enzyme, which fills in the gaps, and DNA ligase to seal the ends. All we had to do was create the cohesive ends, anneal them, add DNA polymerase and ligase, and covalently closed circles would be formed, one half of which would be SV40, and the other half lambda dv gal.

Hughes: That's very clear.

Berg: So, it's very simple.

Peter Lobban's Research on Recombinant DNA

Berg: Actually, Peter Lobban, who was a graduate student in Dale Kaiser's lab, had come up with the same idea on his own. One



Paul Berg: "Arrows denote gaps to be filled in before DNA ligase can close the circle."

could have used G's and C's for cohesive ends, but he also used A's and T's on each end. What's necessary is that they be complementary, that is, form base pairs and join the two DNAs to one another. So this was what we developed in 1970-71, '69.

The Jackson, Symons, Berg Paper, 1972

Berg: A postdoctoral fellow in my lab, who had done his graduate Ph.D. work here in biology with Yanofsky, came over and joined my lab and actually did this experiment. And it took not more than about seven or eight months.

Hughes: Who was that?

Berg: David Jackson. And there was a sabbatical visitor from Australia named Bob Symons. So, the paper is actually Jackson, Symons, and Berg.[1]

Hughes: If you were to single out one paper, would that be the paper upon which the Nobel Prize was based?

Berg: Yes, I think so. If you read the paper, you see that in the discussion it lays out that the ability to join two DNA molecules together allows one to begin to make all kinds of recombinants. We used lambda dv gal, but any piece of DNA would work. We anticipated that some technology would evolve in the future to isolate individual genes, and therefore individual genes could be plugged in.

Lambda dv gal plasmid itself has the capacity to replicate in *E. coli*. So if this molecule is introduced into *E. coli*, it could be replicated and maintained as a plasmid. If put into mammalian cells, we presumed that it would integrate. As it turned out--Stan Cohen was right--we opened up the lambda dv gal at a position that destroyed its ability to replicate. And therefore this molecule would not have replicated in *E. coli*.

Hughes: How did you interpret that at the time?

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[1] D. A. Jackson, R. H. Symons, and P. Berg, "Biochemical methods for inserting new genetic information into DNA of Simian Virus 40: Circular SV40 DNA molecules containing lambda phage genes and the galactose operon of Escherichia coli," *Proceedings of the National Academy of Sciences* [*PNAS*] 1972, 69:2904.

Berg: We didn't because we didn't try to do it.

Complexity of the Berg Recombinant DNA Method

Berg: Now, this technique of making recombinant DNAs was viewed as being complicated and probably beyond the ability of most labs. It was assumed that it could be done only in my lab, probably because we had all the enzymes locally and the expertise in how to use them. And that was certainly correct.

Once we decided that we were not going to try to propagate these recombinants, our critics breathed a sigh of relief and said, "Okay, we've been spared. If Berg is not going to put these recombinants into bacteria, there's no longer a problem."

Hughes: Other people wouldn't be able to do it.

Berg: Couldn't do this, right. David Hogness, one of my colleagues, was the only person who actually used this [method] to construct recombinants with *Drosophila* DNA.

The Cohen-Boyer Recombinant DNA Cloning Method

Berg: But the whole picture changed when it was discovered that when certain restriction enzymes cleave DNA they create natural cohesive ends.

Hughes: So now practically anybody could do it.

Berg: Now anybody could do it. You could buy an enzyme, take two DNAs, cut them, mix them, tie them together and, presto, a recombinant DNA.

Hughes: I gathered from your MIT interview that Asilomar I was originally conceived as a two-step conference.[1] But the idea of having the second conference was temporarily dropped. It was only when the

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[1] Interview with Paul Berg, by Rae Goodell, May 17, 1975, Recombinant DNA Controversy Oral History Collection, Institute Archives, MIT, Cambridge, p.28-29. Berg was interviewed again for MIT, by Charles Weiner, on April 17, 1978.

Cohen/Boyer experiments were disclosed that the need to consider their safety implications arose.

Berg: The implications of the Cohen/Boyer cloning and the ease with which recombinants could be made was taken up by a small group of seven people that met at MIT in April of 1974. That meeting resulted in the so-called Berg letter,[1] or the moratorium letter.

Recombinant DNA Controversy

Concern about Berg's Proposed Experiment with SV40

Berg: We didn't ever try to put the lambda dv gal-SV40 hybrid into *E. coli* because when it was discovered that we were making this molecule, there was concern about putting SV40 into a bacterium that inhabits people's intestines. The concern was that SV40 carries tumor genes, and cancer might be spread through this kind of infection.

So, reports of our work in 1971-'72 created a big furor. Nick Wade wrote a book called the *Ultimate Experiment*.[2] The ultimate experiment was to put the SV40-containing hybrid plasmid into *E. coli*. We decided not to do that because we couldn't be sure that this would not produce a bacterium which could get out of the lab and infect people and possibly populate their intestinal tract with bacteria that carried cancer genes. So, we never did the experiment. We never tested whether it could grow in bacteria.

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Hughes: One of the debates was, could bacterial genes not only replicate but also express protein in mammalian cells?

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[1] "Potential biohazards of recombinant DNA molecules," *Science* 1974, 185:303 (July 26, 1974). The signers of the letter were: Paul Berg, David Baltimore, Herbert Boyer, Stanley Cohen, Ronald Davis, David Hogness, Daniel Nathans, Richard Roblin, James Watson, Sherman Weissman, and Norton Zinder.

[2] Nicholas Wade, *The Ultimate Experiment: Man-made Evolution*, New York: Walker and Co., 1977.

Berg: That's right. That was why we did the experiment. We planned to put this plasmid into mammalian cells. That was not a problem; we could have done that, but that was put off because Dave Jackson left the lab for a job at the University of Michigan. Furthermore, concerns arose and expanded about the safety of working with these DNA molecules themselves. Being infectious, could such molecules escape into the air and get into bacteria? These really were very absurd kinds of concerns.

Berg's Involvement

Berg: But the fact is that I got distracted and got involved in this whole debate about whether these molecules were safe or not. As a consequence, a lot of people who were leaving bacterial work to work on tumor viruses raised the question of whether SV40, polyoma, and adenovirus were a hazard for man.

In response to these concerns, I got involved in organizing a conference--Asilomar I [1973][1]--that most people don't know about. It was held at least two years before the Asilomar Conference on Recombinant DNA [February 1975] to consider possible hazards of working with tumor viruses.

Biosafety at Stanford and the Salk Institute

Berg: Remember, I mentioned that when I came back from Salk, I built a new laboratory, equipped with the latest biosafety equipment, because there was concern on the part of people in my department who were working outside my group about whether they were being unduly exposed to potentially tumor-causing viruses.

Hughes: Did those safety measures pertain at Salk?

Berg: Salk was very loose. They had just these little Plexiglass hoods, and you did all your work in there. Everybody was working on SV40, and they didn't worry about it at all. Generally,

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[1] The publication arising from this conference is: *Biohazards in Biological Research*, Proceedings of a Conference held at the Asilomar Conference Center, Pacific Grove California, January 22-24, 1973, A. Hellman, M.N. Oxman, and R.Pollack, eds., Cold Spring Harbor Laboratory, 1973.

scientists didn't worry about whether working with SV40 was dangerous to them. But then you bring it into an open lab, with a lot of people who are not involved in the work. I don't remember exactly how the word got around and why the technicians and dishwashers at Stanford would have been worried about somebody working with SV40. Perhaps it was at a departmental meeting. I had just become chairman [1969-1974] of the department, and so I was very sensitive to this kind of unrest and uncertainty. To mitigate these concerns, we built a lab with filtered air, negative pressure, laminar flow hoods, in an adjacent building. Everybody was reasonably confident it was safe.

Asilomar I Conference, 1973

Berg: Out of this conference, for which there was a big report and book,[1] there was a recommendation to draw blood every six months from everybody working in a lab and to determine if they had antibodies as a measure of their exposure to the virus they were working with. If you had antibodies, it meant you were exposed to the virus and had been infected. As it turned out, in my lab almost everyone became seroconverted.

Hughes: That procedure arose from the first Asilomar conference?

Berg: Yes. [Indicates photomicrograph on book cover] That's SV40 on the cover, from my slides. [The arrangement of the virus particles] happened to form a question mark.

Hughes: Yes, isn't that striking.

Berg: That's the proceedings of the conference, the papers that were presented and the final recommendations. I made the closing remarks. Essentially what it says is, we don't know whether these viruses are oncogenic for man, and asks what steps should be taken to protect ourselves. We had in mind the problem, How do we proceed in the face of uncertain safety? Do we ignore it, or do we try to do something?

Not too many people know about the Asilomar I conference proceedings. For example, here are designs of the kinds of hoods

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[1] *Biohazards in Biological Research*, Proceedings of a Conference held at the Asilomar Conference Center, Pacific Grove, California, January 22-24, 1973, A. Hellman, M .N. Oxman, & R. Pollock, eds., New York: Cold Spring Harbor Laboratory, 1973.

that laboratories should construct. We brought in people who were in charge of biosafety at the NIH, and they described how rooms should be arranged and so on and so forth--facilities and equipment available for virus containment.

Hughes: Was there overlap of the people who attended Asilomar I and II?

Berg: Oh, yes. There were many of the same people who are in here [referring to Asilomar I book]. Here's a chapter on laboratory hazards from aerosols. Those people were at Asilomar II, or they were on the RAC [Recombinant DNA Advisory Committee], or the guidelines called on them to provide information about safety. Now that I look at this list of attendees, a good many of the people were also at the Asilomar II conference.

One of the steps we proposed was to initiate a prospective study, to periodically collect blood samples from people who were working in the field and store them. We still have them, frozen away. The purpose was to keep track of anybody who developed cancers and to check with their prior exposure. Working with laminar flow hoods, in negative pressure labs were also amongst the recommendations that came out of this conference.

These procedures set the stage for entering into an era where we were going to work with infectious organisms whose complete properties couldn't be predicted. Clearly, as we began to manipulate viruses and make mutants, there was a possibility that we might make something that would be more dangerous to the workers as well as to the people around us.

The Moratorium Letter and the Meeting at MIT

Berg: Some of the people who were at the MIT meeting had also been participants in the Asilomar I meeting.

Hughes: Do you remember who they were?

Berg: Richard Roblin was one; Jim Watson, Dave Baltimore, Norton Zinder, were also at Asilomar I.

Berg: The reason Roblin was invited to the meeting was because he had written an article in *Science* about gene therapy which focused on manipulating mammalian cells by virus infections. So, he was invited to come to that meeting.

Hughes: Why was he provoked to write that article?

Berg: Well, the whole notion of genetic engineering of animals was not new. Josh Lederberg had speculated on it earlier. There were a number of science fiction scenarios; in fact, oftentimes gene therapy was cited as justification for doing genetics.

Hughes: Arthur Kornberg testified in the Senate in 1968 on genetic engineering.[1]

Andy Lewis and Natural Adenovirus-SV40 Recombinants

Berg: A man named Andy Lewis, working with adenovirus at the NIH, had raised concerns about adenovirus-SV40 recombinants. Adenoviruses are big viruses of about thirty thousand base pairs. What Lewis found is that cells that were coinfected with SV40 and adenovirus produced recombinants which had replaced part of the adenovirus with SV40 sequences.

These were called adeno-SV40 hybrids, and they propagated as viruses. But they were defective; generally, when adenovirus genes are replaced by SV40 genes, the recombinant virus can't multiply. But Lewis discovered a class of adeno-SV40 hybrids which were nondefective and could replicate. He was very concerned, because we're all infected with adenovirus and if SV40 got out, it might form adeno-SV40 hybrids which could propagate and spread SV40 genes. Consequently, a very big part of the Asilomar I meeting addressed the concern about what to do about these adeno-SV40 hybrids.

Hughes: What would be the purpose in an evolutionary sense for SV40 and adenovirus to be able to join?

Berg: There's no disadvantage or advantage. It happens accidently.

Hughes: That doesn't happen very often, does it?

Berg: No. But here they'd cloned these out; they had pure populations of these adeno-SV40 hybrids. People found them very useful and wanted to use them as tools.

Hughes: So you were creating an artificial circumstance.

Berg: That's right. Andy Lewis to this day is concerned about such events. There was just a conference at the NIH, "Is SV40

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[1] See the oral history with Arthur Kornberg, cited p. 8.

Tumorigenic for Man?" Because a number of people have come down with tumors with SV40 genes in them. They're saying it just took a long time for this to happen. One of the sessions at this recent meeting was called "Hazards Associated with Modern Research Methodologies."

James Watson's Stances

Berg: Coming out of the Asilomar I conference, Jim Watson decided to forbid the use of feline leukemia viruses or cats at Cold Spring Harbor because he was so worried about whether these viruses could cause cancer in man.

Hughes: Yet one of the amazing things that happened at Asilomar II was that Watson changed his tune about the dangers of research with tumor viruses.

Berg: That's right. If you're cynical, you might speculate on why. Jim was at the MIT meeting that came out with the Berg letter. He was absolutely supportive, insisting that we had a responsibility to warn the general public and scientists about the potential dangers of cloning.

One of the consequences of the Asilomar II conference was to mandate that research could proceed only under conditions which guaranteed the organisms you were working with would not escape from the lab. I had already invested in building a P3 [physical containment 3] laboratory that minimized that risk, and it was expensive. Jim was now pushing working with tumor viruses, SV40, at Cold Spring Harbor, and he realized that if such safe facilities were required he would have to invest and build much secure facilities there. Jim could certainly calculate what the cost would be to build the kind of facilities being proposed at Asilomar, and he knew that not doing so would impede their ability to compete. I suspect he also came to the conclusion that we had been rash at the first meeting in presuming risks that he now thought were unreasonable.

Hughes: At one point you accosted him over his change in stance. Can you remember what he replied?

Berg: Jim never would have admitted that there was an economic component to his argument, or his ability to compete. I think what he said, and he may have felt that honestly, is we made am mistake in the Berg letter by concluding that there was great concern.

Hypothetical Risks

Berg: I think if you reflect, as we all did later, on the basis for this concern, it was all hypothetical. There was not strong reason to believe that what we were doing would be dangerous. It sounded dangerous. We wondered what might be the consequences if you put genes that confer resistance to antibiotics into bacteria that infect man; you'd prevent the use of antibiotics that cure whatever that bacteria caused. That sounds pretty worrisome, and we suggested that such experiments should not be done. Second, putting genes that specify toxins into bacteria that could inhabit man should also not be done. But then when you come down to the rest of it, it was pure hypothesis. We could imagine you might inadvertantly pick up oncogenes from mammalian DNA, incorporate them into plasmids, and put them into bacteria. Well, so what? As it later turned out, even if you do, it doesn't make any difference. It turns out it's safer to work with oncogenes that way then it is to try to work with the viruses which carry them.

Hoof and mouth disease is one of the serious virus infections, so all work on hoof and mouth disease is done on Plum Island, an island off the coast of Long Island. But you can fragment hoof and mouth disease [virus] and clone its genes, and it's quite safe in that form. Hepatitis can only be grown as DNA segments, as plasmids. We began to realize that in some ways you could say it was safer to be using recombinant DNA.

Nonmicrobiologists and Research on Infectious Organisms

Hughes: Asilomar II was February, 1975. You began working with SV40 in 1971?

Berg: Nineteen sixty-nine.

Hughes: Do you remember when Lewis began working with adeno-SV40 hybrids?

Berg: Yes, it was around '71.

Hughes: So, by Asilomar II, there was five or six years of research experience with--

Berg: Not with recombinant DNA, since the Cohen/Boyer experiments were done during '73, '74.

Hughes: I'm calling what you had done recombinant DNA, and in a sense, Lewis's research, even though I know that the adeno-SV40 hybrids were not a deliberate creation. What I'm trying to assess is how much experience you collectively had had with recombinant organisms by Asilomar II. And it seems to be roughly six years of research.

Berg: Right. But only a very small number of people had had that experience. Remember, the [Cohen-Boyer] recombinant DNA breakthrough made it possible for anybody to do anything. This was recognized, and was one of the driving forces for the moratorium letter. There was a lot of people who had no experience working with potentially pathogenic organisms and they would be moving into the field. In fact, it was common for biochemists and molecular biologists who had been working with *E. coli* to grow up *E. coli* in five-gallon jugs and dispose of some down the drain. *E. coli* was viewed as innocuous, and some people might have volunteered to drink it. But microbiologists and bacteriologists knew that *E. coli* was not innocuous.

Everybody was pipetting viruses by mouth; that's the way we worked. The reason people who worked with viruses were seroconverted was because when you suck up a column of a solution with a virus in it, you have a vapor, an aerosol at the top, and that gets taken up [in the digestive track].

We realized that in fact the technology now had changed the way people were going to do things, and most of those who were going to be working in the field were totally inexperienced and unaware of the most trivial safety measures. And so part of the rationale for the letter was to bring this to people's attention.

Hughes: I know from talking to microbiologists that they had scathing remarks to make about the laboratory safety techniques of non-microbiologists.[1]

Berg: Microbiologists used a loop. I don't think a biochemist knew what a loop was made for.

Hughes: This confluence of disciplines, that we talked about, has so many ramifications. One of them is bringing in disciplines that don't have a long background in working safely with pathogens.

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[1] For example, see Edwin Lennette, *Pioneer of Diagnostic Virology with the California Department of Public Health*, Regional Oral History Office, The Bancroft Library, University of California at Berkeley, 1988.

Berg: That's right. If biochemists broke and spilled an important experiment, they'd suck it up without thinking, even if it was something dangerous. Their goal is to save the experiment.

Achievements of the Research Moratorium

Berg: Well, if you were working with really hazardous things, you'd say that kind of behavior is unacceptable. But if you were working on problems at open benches, you don't know how such people think about risks. What does it take to do so? What kind of ethics or whatever does it take for somebody to say, wait a minute, you don't want to do something so stupid that it's dangerous to yourself, to your family, to your co-workers. It doesn't take any higher calling for somebody to say, "Hey, wait a minute, we ought to think a little bit about what we're doing to see whether it's safe."

Hughes: And that's what Asilomar II and that complex period of history did?

Berg: That's right. Today, there's no question that it impeded some research. But my argument is that impedance actually benefitted the research in the long run. For one, I think we didn't generate the kind of public opposition that could have easily stopped the research. Because we took the initiative, and brought attention to the problem, and tried to deal with it, people accepted that we were conscientious, well-meaning, and responsible.

Second of all, I don't know how much work on cloning human genes could have been done right from the beginning. We didn't know how to walk, no less run. We barely could crawl. When the Asilomar constraints said you can do this under these conditions and those conditions, people learned how to do the cloning under safe conditions. People learned to adapt so that now nobody ever used mouth pipettes; people worked in hoods; people had to take care. And before you knew it, it was possible. The technology had improved. People's approach to things had changed. You could now go after human genes. So, I don't think that we lost a lot by saying you couldn't clone human DNA for the first year or two. But it certainly prevented people, or called attention to the fact that you had to think about what you were going to do before, not after. And you had to think about, was it sensible? Was there any kind of a risk? Could I learn what I want to learn another way?

Hughes: Well, there were some examples of things that had gone wrong, particularly in Britain. I understand that they colored the Ashby group's considerations. What was it? Smallpox?

Berg: Yes.

Hughes: There had been some recent deaths in a British laboratory. As you say, if the research momentum had continued unabated--

Berg: Somebody would have done a dumb experiment.

But you also have to remember that there were scientists, who had a very different perspective--Science for the People kind of thing. They were primarily left-leaning people. The Vietnam War spawned a whole lot of people to be very suspicious, not accepting. There were claims being made about certain genes predisposing to criminality. And this group in Boston [Science for the People] reacted to that.

Had we not called attention to what we were doing [recombinant DNA research], they would have. I believe they would have said to the public, "Look at what these guys are doing; this is really dangerous stuff. It's the first step to genetic manipulations of man," and blah, blah, blah, which is what they tried to do. But given that we had raised the safety issue first and tried to deal with it, I think their message was blunted. And while they were harassing us much of the time and were successful in some places, Cambridge and so on, in the end they didn't win. And the science moved forward.

So as I look back on the period, even though we were wrong--wrong is probably not the right word; certainly our assessment of the potential risk was incorrect--by calling attention to it, I think the whole thing was better off in the long term. The science that has come out of it has just been absolutely mind-boggling. And so that's what in the end will justify it.

Transduction

[Interview 3: September 30, 1997] ##

Lysogeny and Transduction

Hughes: Dr. Berg, my goal today is not to talk exhaustively about the biohazard issue, which has been well-covered in the historical

literature.[1] Instead, I want to focus on the science. What were the technologies that made the recombinant DNA work possible?

Berg: Well, there were really two lines of investigation that were important. I think I ended my last interview talking about lysogeny. Lysogeny is a phenomenon in which viruses that infect bacteria can integrate their chromosome into the chromosome of the infected cell. And then they are maintained as if they were a normal part of that organism's chromosome. They're replicated each cycle. The cells are perfectly healthy. They reveal some new properties as a result of having acquired the virus information. But with low frequency, this mutually acceptable state breaks down and the viral chromosome pops out of the cellular chromosome and replicates, killing the cells and producing the virus. That state is referred to as lysogenic, meaning that the cells, while they're perfectly normal and grow perfectly well, enter a state after the virus is activated, and the virus kills the cells by lysing them.

Now, if you ask, what is the virus that comes out? Is it exactly the same as the virus that went in? And, 99.999 percent of the viruses that come out are exactly the same as those that infected the cells originally. That is, they are excised from the cellular chromosome perfectly accurately. But, occasionally, they actually come out with some of the adjacent cellular genes. And so, the virus that comes out now is different than what went in. It has lost something of its own chromosome and picked up some of the cellular chromosome. Such viruses, when they infect the next cell, will be able to transfer genes from the original cell they were in to the new cell they infect. That process, called transduction, was discovered many years ago by Norton Zinder and Joshua Lederberg. So depending upon where the virus integrates, it's able to pick up genetic information of the cell closely linked to it.

Other Forms of Transduction

Berg: There's a second phenomenon of transduction in which another virus, a different one, goes in and infects the cells and kills the cells. And, in the act of killing the cells, it literally pulverizes the cellular chromosome into bits and pieces. In the

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[1] See, for example: Susan Wright, *Molecular Politics: Developing American and British Regulatory Policy for Genetic Engineering*, 1972-1982, Chicago: University of Chicago Press, 1994.

packaging event, which leads to the production of new virus particles, these bits and pieces of cellular chromosome are inadvertently packaged into the viral particle. As a consequence, a population of viruses is produced which carries different pieces of the cellular chromosome as well as their own. If you looked at these virus particles, you couldn't tell the difference as to whether they had their own chromosome or whether they carry pieces of the cellular chromosome. They infect cells; they bring in this new genetic information. And this genetic information can then replace what is present in the cell. And so, you now get a new genetic property. This process is referred to as transduction.

So, there are two kinds of transducing viruses. One of them is what we call lysogenic, the virus goes in, integrates, stays there for however long, spontaneously pops out, most of the time accurately. Occasionally, it picks up cellular genes alongside it. And if you know where the virus goes, you can actually then transfer genes that are adjacent to the site of integration.

Hughes: This was all well known?

Berg: All well known.

Hughes: By when would you say?

Berg: I was doing this in conjunction with Charles Yanofsky, who's a geneticist. This is the technique of modifying bacterial cells and introducing new kinds of genes in them. For that purpose we used bacteriophage lambda or phi 80. Those are two kinds of phages. They each integrate in different places in the host chromosome. We used the phi 80 phage because it often could carry the genes that controlled tryptophan synthesis, and we were studying tryptophan mutants. We could transduce genes from one cell into another and create cell lines that had useful genetic properties for our experiments.

The lambda phage goes into a different part of the *E. coli* chromosome, and it carries different sets of genes when it comes out. The P_1 phage is different because it picks up random pieces of the cellular DNA. That's also very useful because in any population, a virus that comes out of an infection will carry genes that you're interested in studying. Using P_1 you can transfer any genes.

So, the important conceptual point is, there was a way to transfer genes from one cell to another, and it had proven to be extremely useful and powerful in setting up experimental systems for genetic studies.

Hughes: Is this a potential evolutionary mechanism?

Berg: Usually these viruses are quite specific for the cells they infect. So, it isn't that they spread their genes throughout a population. But certainly within a certain group of organisms, genes are flowing back and forth. No question about that. I suppose if you put a selection on, that is conditions which favor the growth of cells that contain one set of genes, those will have preferential growth, and the others will die out.

Hughes: So, there was a natural mechanism that you took advantage of.

Berg: That's right.

Hughes: What couldn't you do with it experimentally that you hoped to do? Why devise an artificial laboratory mechanism for transferring genes?

Berg: Because you could, in fact, use conditions that selected for just the kinds of organisms you want. By doing the transduction in several ways, we were able to adapt that natural phenomenon to our advantage. Now, this was true of bacteria and bacteriophages. I would attribute a very large part of the burst of genetic knowledge during the 1960s to the astute use of these bacteriophages. That certainly helped. Another thing is, once these viruses come out, you can isolate the ones that carry these cellular genes, and then you begin to analyze the sequence.

In one case, it was possible to sequence a large part of one region of the bacterial chromosome because it was now highly enriched in the bacteriophage DNA. After all, the viral chromosome was tiny compared to the cellular chromosome, so you get an enormous enrichment. If you harvest the virus, you've now looked at a small portion of the cellular chromosome and can analyze that. And that was done.

Transduction of Mammalian Cells

Hughes: You said that you had a natural system that you were taking advantage of. What I see coming next, with recombinant DNA work, is that you created constructs for scientific purposes. No longer were you just taking advantage of what nature had already provided.

Berg: That's right. But the important thing is to realize that in mammalian cells, there was no such natural system. No viruses

were known that could transduce genes to mammalian cells. Well, let me put it this way. When I went to the Salk Institute for a sabbatical, it was to learn about how to work with mammalian cells. The motivation formed from the fact that viruses had been so influential and important for the development of the molecular biology of microorganisms that maybe studying the interaction of animal viruses with animal cells would give us the same kind of insights.

The polyoma virus I studied has a very small chromosome, only five genes. So, if you wanted to study how a cell expresses its genes, instead of looking at how it expresses its own genes, why not look at how it expresses the virus genes once they enter the cell--a much easier system. So, when I went to Salk, it was to begin to understand something about how these tumor viruses multiply in a mammalian cell.

We chose the tumor virus because it too mimics, to some extent, lysogenic viruses. It can integrate into the cellular chromosome. And we thought, well, perhaps under certain conditions it could come out and carry with it genes that are adjacent to where it had integrated. During that year, we had a lot of success studying some interesting features about polyoma infection of mouse cells.

One of the things that others discovered was that there were virus particles coming out carrying cellular DNA. That looked like the P_1 phage. So the question was, could we in fact use such viruses in the same way that the bacterial people had used P_1 as a way of transporting genes into mammalian cells? The idea was to grow the viruses in one kind of cell, then take the population of viruses that come out and infect another population of cells, and ask whether interesting genes were transferred from one to the other.

Hughes: How at that time did you distinguish viral from mammalian DNA?

Berg: We could label the DNA of the virus, and we could follow its transactions, if you will, once it entered the cell. Two, once we have the DNA of the virus, we can use that DNA as a probe. When the virus enters the cell, it's transcribed and makes messenger RNAs. If we had the pure DNA, we could use the DNA to detect those messengers. But since isolated genes from mammalian cells were not available, there was no way to follow the transcription or expression of mammalian genes.

Remember that the mammalian genome is larger and more complex than the bacterial genome, and the amount of DNA is a thousand times greater than is present in a bacterium. So, the

idea of being able to follow the expression of mammalian genes was very complicated. There were a lot of puzzles. For example, when people tried to look at something that was supposed to be the equivalent of messenger RNA, they found an enormously complex mixture of RNAs of varying sizes; most of it never ended up as messenger RNA. That was a real puzzle. In bacteria, you could measure transcription. An RNA molecule is colinear with the segment of DNA from which it is transcribed. But in mammalian cells there was this mess, a real mess, 98 percent of which never makes it into the cytoplasm.

Hughes: You didn't find that intimidating?

Berg: No. Some people did. Kornberg tried to persuade me not to get involved in such a messy system; "You're wasting your talent," he told me. He was really very, very critical of the decision to enter this field. However, I was convinced that we were beginning to know a lot about the bacterial genetic system--how it's expressed, how it's replicated, and so on. There were elaborate theories of regulation, of the messenger RNA concept. One had to stop and ask oneself, Is all of this unique to the bacterium? Is this the way things happen in higher organisms? Well, I wanted to learn if this whole system of messenger RNAs, transcription, operators, repressors, polymerases, and so on, existed in mammalian cells. How do you begin to study mammalian cells? That is intimidating. The virus was the key. The virus was the way to get into that system and simplify it.

Hughes: So, the virus approach made it acceptable to you to do this very complicated, risky research?

Berg: Right. In fact, it turned out to be correct; the virus was the simple way to be able to look at it. And people just went to different viruses. We began to work with SV40; other people worked with polyoma, adenovirus. But the virus was the entree into studying how mammalian cells deal with a piece of DNA. Use a small piece of DNA [the virus] so that you can distinguish it from the cellular DNA.

During the year at the Salk it was discovered that viruses coming out of the infected cells seemed to be carrying some mammalian DNA sequences. We thought, perhaps is this the analog of the P_1 transduction system? If you make the calculation, you soon realize that SV40 can only package about 5,000 base pairs of DNA, whereas the bacteriophages that we were using before could package 50,000 b.p.

Hughes: What are the limitations?

Berg: The limitations are the size of the viral capsid or shell capsid. The capsid has to be built, and only so much DNA can be stuffed in it. So, 5,000 base pairs of DNA go in. Now, if you ask, given the size of the mammalian genome, 3 billion base pairs, a 5,000 base pair segment of it is a very, very small fraction of the total. So, the question is, any one virus particle could only contain an infinitesimally small amount of the cellular chromosome. If you were looking for a particular gene, it would be very rare. And if it was very rare, you would have to have an incredibly powerful way of detecting its transfer and ultimate function. And so we quickly realized it was hopeless to use this as an analog of P_1. Of course, we knew nothing about the likelihood of being able to incorporate a whole gene. We didn't know the size of mammalian genes. Now we know it would have been impossible.

At that time we thought mammalian genes were about the same size as bacterial genes. You might have been able to package them but it would have been so rare that only maybe one in a billion particles would actually contain the gene of interest in functional form. And then you would have the job of finding a needle in the haystack. You'd have to have an enormous population of mammalian cells infected by an enormous population of virus particles in order to detect the transfer of one gene.

Hughes: So, detection is really key, isn't it?

Berg: Logistics, detection, and the packaging limitation in the virus were all things that precluded what we had in mind, namely that you could transfer genes using SV40 viruses as vectors. If you had viruses that could carry much more DNA, you might have had a chance.

More on Recombinant DNA Science

Construction of Recombinant DNA Molecules to Study the Mammalian Cell

Berg: So we said, okay, that's not going to work. Can we construct DNA molecules which in fact use the virus chromosome? Let's just attach foreign pieces of DNA, any genes we might ultimately want, to the viral DNA. At the time, you have to remember, nobody had isolated any genes. I believed that that would eventually be

achieved. People would find ways. One hint that this was likely was that certain classes of genes have physical properties which allowed them to be separated from the bulk of cellular DNA.

Don Brown, at the Carnegie Labs in Baltimore, was able to isolate pure ribosomal RNA genes, because they have an unusual buoyant density. That is, if you put them in a centrifuge in a gradient of salt concentration, the ribosomal DNA genes separate from the rest of the DNA. And, you can purify them that way. Nobody had isolated any other genes, but it wasn't too far-fetched that there would be a way to isolate individual genes sometime in the future. So, on that premise we said, "We need to develop a method for attaching any piece of DNA to SV40 DNA."

Hughes: Was your premise based on the fact that you had a technique which could be developed further, or was it an expression of optimism that science will find the answer?

Berg: There was a certain amount of optimism, faith, however you want to refer to it. But, in this particular case, I think what we were asking was a question that was wholly within our own domain. Could we devise a way to attach any piece of DNA to SV40 so that, if cells were exposed to this recombinant DNA, they would take it up. If so, there would be a way of introducing this DNA into a mammalian cell.

Now, this piece of DNA could be nondescript, that is, from any source. From a technical point of view, all we needed to do was to learn how to attach two pieces of DNA. The faith and optimism was that down the line people were going to find ways to prepare genes that would be interesting to put into cells.

So, for our purposes, we just started with a piece of bacterial DNA which had three genes whose properties we knew something about. And if these were attached to SV40 introduced into mammalian cells, we could test if bacterial genes could function. That was a totally unanswered question. As we know today, they would not have been functional. But, we didn't know that then. So, the question was, if you could in fact take genes from other organisms and attach them to SV40 and piggyback them into the mammalian cell, would they function? And if they functioned, could you learn something interesting about the mammalian cell?

Bacteriophage with Cohesive Ends

Berg: The question was, How do you attach two pieces of DNA? So, now comes the second bit of information that was standard lore. You asked me about the precursors. People had been studying lysogenic bacteriophages, e.g. bacteriophage lambda and phi 80. The DNA in phage lambda is about 50,000 base pairs long, and it exists in the virus particle as a linear, double-stranded DNA. What was discovered is that these viruses had unusual ends, sticky ends. They had protrusions of single strands from each end. These single-strand ends are complementary to each other so that, even though the DNA from the bacteriophage is clearly linear, the DNA circularizes when subjected to very simple conditions. They circularize, and they circularize because the two single strands come together and form double strands. These linear molecules can also form long chains, because they attach end to end through the cohesive ends. And that's the origin of the term sticky ends or cohesive ends.

Hughes: That was work done here at Stanford?

Berg: Well, some of that work was done here by Dale Kaiser, who was our principal person in bacteriophage and one of the major workers in the field of lysogeny. Alfred Hershey, who was then at Cold Spring Harbor Laboratory, was the initial discoverer of cohesive ends. It was learned later that this class of lysogenic phages had cohesive ends. But the ends are all different. So, if you take lambda phage and phi 80, they will not join to each other, because their ends are different. Sticky ends are important in the reproduction of the virus. The virus goes in as a linear molecule, and once inside the cell, it circularizes and the nicks between the ends get closed. And then it functions as a circular DNA molecule.

Creating Artificial Cohesive Ends

Berg: So, the concept of sticky ends already existed. If you want to join two different molecules together, it doesn't take a genius to figure out that if you can create artificial ends that are complementary to each other the two DNA molecules will come together. Right? No big deal.

So, if you put tails of A on one piece of DNA and tails of T on SV40 DNA, and mix them, the A's and T's will form double

helices, and the two molecules will come together. Each DNA cannot join to itself, only to one with complementary tails. We could have used G's and C's, but A's and T's were easier to add.

We already knew how to add tails onto DNA molecules because there is an enzyme that had been described which is present in calf thymus; it has an interesting physiological function, but that was not known at the time. It is a DNA polymerase, but a "dumb" DNA polymerase. It doesn't need a template. If you give it any one of the four deoxynucleoside triphosphates, it will add the nucleotide on to the end of the DNA molecule, producing long chains of the same nucleotide. So, if DNA molecule A is mixed with deoxyATP and this enzyme, long polymers of A's are added onto the two 3-prime ends of this DNA. And if you do it with deoxyTTP, long chains of T's are added. By regulating the time of the reaction, you can add, on average, about 100 A's or T's onto each end.

Hughes: Who worked out that procedure?

Berg: The enzyme was found by Fred Bollum and it is called nucleotidyl terminal transferase. This enzyme cannot make DNA molecules de novo from deoxytriphosphates; it always needs a primer end. And, we knew that. Therefore, if we took a DNA molecule, its 3-prime ends served as primers to polymerize onto the ends. Then adding T's to the ends of a second DNA, they would come together to form circles.

Enzymatic Sealing of DNA Circles

Berg: Now, the dA and dT tails are uneven because there's no way to get them precisely the same length. When the two DNAs are joined, there are gaps at the join. Kornberg's DNA polymerase was the perfect thing to fill in gaps; we knew that. We knew that once we made circles they would have gaps, but these could be filled with DNA polymerase. You just add all four deoxytriphosphates, and the enzyme fills in the gaps. And if you add the enzyme DNA ligase, which had also been co-discovered at Stanford by Bob Lehman, the ends become covalently joined. So by taking these two separately prepared DNA molecules, mixing them, waiting a few minutes until they anneal, adding DNA polymerase, four deoxytriphosphates, and DNA ligase, then you have a recombinant DNA.

The recombinant DNA we made involved joining SV40 together with the piece of DNA that contained three bacterial genes. The

reason we could use these three bacterial genes is because they had been picked up by lambda by this kind of inexact excision.

Biochemistry Department Contributions

Hughes: You had all the ingredients to perform this experiment right there in the biochemistry department?

Berg: In a refrigerator which I had complete access to. That's a terribly important point. I've made that point many, many times over. It's a reflection of the kind of atmosphere that we had in our department, the kind of relationships which we had with each other, which was completely giving and open. It wasn't competitive. It wasn't secretive. I had access not only to all of the information that would ultimately be needed to do this experiment, but in addition, the materials were accessible. I didn't have to stop to make these enzymes. We made some. But the point is they were all there and the expertise for how to use them.

Hughes: Very important.

Berg: Very important. And so, the actual accomplishment was quite straightforward.

Replication and Expression in the Host Cell

Berg: Now, we intended to introduce these recombinant DNA molecules into mammalian cells. And the question was, would the bacterial genes function that were introduced along with the SV40 DNA? Before we got the chance to do that experiment, it was recognized that the piece of bacterial DNA we had used also was capable of replicating in bacteria. So in principle, this recombinant DNA could also be put into bacteria to determine if the recombinant DNA would replicate. And would the SV40 genes be expressed in bacteria?

Hughes: Was that a question that you asked at the outset?

Berg: No, that was not a question that was in our plans. Some people, particularly Stan Cohen, thought that was what we were aiming to do.

Concern about Potential Biohazards

Janet Mertz at the Tumor Virus Course

Berg: But in point of fact, what happened, and this is part of the recombinant DNA story, is that one of my students, Janet Mertz, went off to Cold Spring Harbor during the summer of 1971 to take a tumor virus course. As a student in the course, she was asked to give a seminar on what research she was doing, and she described what we were doing. That aroused enormous anxiety.

Bob Pollock, one of the instructors in the course, got all exercised about the possibility that we would be putting genes from a tumor virus into a bacterium which normally inhabits humans. Bob called me from Cold Spring Harbor asking, "Why are you doing this crazy experiment?" He was referring to the possibility of introducing recombinant DNA into *E. coli*. That wasn't in our mind at all. During all of the furor, concern was raised that just making these molecules in the lab might allow them to come in contact with bacteria, and these would spread a plague of cancer.

Berg's and Lederberg's Reactions

Hughes: According to the accounts, Janet Mertz called you from Cold Spring Harbor. Can you remember your first reaction?

Berg: My first reaction was, this is stupid; this is bunk. I thought it was outrageous. Then, as we talked a bit, I kept trying to think of ways to sort of fend off, or even respond to, the concerns by saying this, that, or the other thing. By that time, it was clear that I was being forced to consider what might happen in an experiment that we hadn't even thought about doing. We kept talking about different ways to prevent what was of concern. But, given how paranoid the concerns, I began to ask myself if there was a small possibility of risk. And if there is, do I want to do the experiment?

I remember going to talk to Josh Lederberg because he had already been talking about making germ warfare weapons, using the new molecular genetics, as we do with the bacteria. Nothing had been talked about using mammalian cells or animal viruses or anything. And Josh, interestingly enough, was very, very conservative. I mean, he was not about to say, "Go ahead and do

this experiment." Instead he said, "There's certainly the possibility, and you have to take responsibility." At that point I stepped back and asked, Do I want to go ahead and do experiments which could have catastrophic consequences, no matter how slim the likelihood?

Hughes: Yes, and, as you said last time, you had already had concerns in this general area. You were the convener of Asilomar I.

Berg: And Pollock was one of the key people in that too; in fact we had been its co-organizers.

Hughes: Hadn't Lederberg been involved with advising on space probes and the potential danger of bringing foreign microorganisms to this planet?

Berg: Yes, absolutely, it was in the sixties.

Although my first reaction to Pollock's call was outrage, I had many conversations with him by phone. Since I knew Bob quite well and thought we had a very good relationship, I couldn't just fluff off what he was concerned about. Then I began to talk to still other people, a fellow named Ted Friedman who had also been influential in organizing the first Asilomar conference. Ted had been a strong advocate of ethics in science. I was barraged by hypotheticals, and they were all hypotheticals. There was certainly no evidence that if a bacterium carrying genes that produce cancer in man got into your gut that this would inevitably cause cancer. There was certainly no indication that this was the case.

Putting the SV40 Experiment on Hold

Berg: I guess in the end I finally said, well, let's step back and ask, is there another way to answer the question for which this experiment was designed? The experiment was designed to ask, can we get genes into mammalian cells, and if we do, do they function? The question was, could you set up a different kind of experimental system which would not create a viable organism that could be spread. And so, we put the experiment on hold.

I suspect we would not have put the experiment on hold had Dave Jackson not taken a job and gone off to Michigan. The fact is that he had accepted this job and was due to go to Michigan even before he finished the recombination experiment. And it was only because I called his new chief and said I was not going to

let him go; we're going to chain him to the bench. He had to finish the experiment before he left because I knew that once he left, the chances of him continuing it in a new lab were small. And so, he stayed. Once we got the paper written, he left.[1]

The next step would have been to introduce this DNA into mammalian cells and do the experiment. And that was trivial. I mean, it would have been done. But, with all of the furor that got raised, we just decided, Let's hold off.

Hughes: So, there were two things that were stopped. There was the transfer of the recombinant construct into mammalian cells and also into bacterial cells.

Berg: That's right. The concern was primarily about the bacterial cells. Nobody raised any serious objection to putting the construct into mammalian cells. They raised the objection that just having the DNA and bacteria in the lab, working with them, that DNA does float around, that there was a small chance that it would be taken up by bacteria. There were a lot of ridiculous things.

Hughes: But how ridiculous were they really at the time?

Berg: They were ridiculous only in the sense that there was no evidence that there was a risk. First of all, SV40 has infected humans. SV40 was discovered in the [Salk] polio virus vaccine, so millions of kids were inoculated with SV40 and produced antibodies to SV40, and there's no history of any tumors that have ever arisen. This was documented at the first Asilomar meeting. That was one of the arguments why we didn't think working with SV40 was so dangerous.

But people would say, well, but this SV40 is coming into a human in a new way. It's coming in bacteria that will essentially fill the intestinal track with cells that are dying and spilling out DNA molecules. DNA molecules are easily destroyed, especially in an environment like the gut. What's the probability that a DNA molecule will survive that? What's the probability that DNA will be taken up by an epithelial cell in the gut? All these were unknowns. But it was very difficult in the climate that I described where we were already thinking about potential risks.

Second, I think I mentioned to you in one of our earlier interviews that many of us had adopted a much more social

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[1] D.A. Jackson, R.H. Symons, P. Berg, "Biochemical method for inserting new genetic information into DNA of Simian Virus 40," *PNAS* 1972, 69:3365.

conscience. All of us were of a like liberal mind, and we felt that ethics and responsibility in science were important. The nuclear weapons program was exploding. All of us felt an obligation not to do something that was involved with germ warfare and things of that sort. So, there was a sense of wanting to do the right thing. And not doing the wrong thing because you were selfish, because it was your experiment, your idea, and you were going to pursue it hellbent; no matter what anybody said, you were going to do your experiment. It was much more a matter of a social thing: We ought to talk this out; we ought to think this through.

Well, the thinking through created this brief interval after Asilomar I when nothing happened. And as Nick Wade and other people said, "The world breathed a sigh of relief." The ability to construct such DNA molecules was thought to be so technically challenging that it could only be done in Paul Berg's lab or at Stanford. And if they've decided not to do this research anymore, we're safe. It's interesting how people misunderstood how difficult it was to do. But what was true is people recognized that not many places had access to the enzymes, and the skill, and the experience that was present in our department. And so they said, "If he's not going to do it, nobody else is going to do it." This was 1971. Less than two years later, we discovered that one of these restriction enzymes makes these cohesive ends. We didn't have to synthesize poly-A tails and poly-T tails.

Contributions to Recombinant DNA Science

David Jackson's Opinions

Hughes: David Jackson named four lines of science which contributed to recombinant DNA technology. One, studies of DNA structure and physical chemistry.[1]

Berg: We knew that single strands that have complementary sequences form duplexes. I didn't regard that as a major thing, but it clearly was implicit in everything we did.

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[1] David A. Jackson, "DNA: Template for an Economic Revolution," in: *DNA: The Double Helix. Perspective and Prospective at Forty Years*, Donald A. Chambers, ed., New York: New York Academy of Sciences, 1995, pp. 356-356-365.

Hughes: Two, the enzymology of DNA synthesis and degradation; we talked about that. Three, bacterial, phage, and plasmid genetics. You haven't spoken about plasmidology.

Berg: Because we weren't interested in plasmids. [laughter] We didn't even know anything about plasmids. For us, the plasmid was a viral chromosome.

Hughes: But plasmids are going to feed into [Cohen-Boyer] recombinant DNA.

Berg: Oh, yes, that's the next stage.

Hughes: Four, bacterial restriction and modification systems.

Berg: But even that wasn't implicit in anything we did.

Hughes: I don't think Jackson was writing specifically about what the Berg laboratory was doing.

Berg: In our case, the two things that we knew were, one, that in bacterial systems, viruses could pick up genes. We considered whether that was also a possibility in mammalian systems. And the second was cohesive ends. Cohesive ends were a way to join DNA molecules together.

We didn't need to know anything about restriction enzymes because, in fact, the way we opened SV40, which is circular, was to cut it with an old fashioned enzyme, DNase, which made random double-strand breaks. That allowed us to produce a permuted population of linear molecules. They could be opened up anywhere. And that means what you're inserting could go into any part of the SV40 DNA molecule. Because, once we opened it up, we could add the tails onto the two ends. We didn't need restriction enzymes. So, all of the things that Dave mentions contributed to the evolution of the second stage.

Hughes: What you mean by the second stage is--

Berg: Cloning.

Hughes: Were there methods for joining two pieces of DNA?

Berg: No.

Hughes: Well, Sgaramella was in Khorana's lab. There was a paper published [1970] on a ligase-mediated form of DNA joining.[1] How does that work fit in?

Berg: What Khorana did was set out to synthesize a gene chemically. It was a gene that was about a hundred base pairs long. What he decided to do was to make single-stranded [DNA] pieces. Now, if you make a second single-stranded piece that overlaps it, the two will form a duplex where they pair and single strands at the ends where they don't pair. And then he made another single-stranded piece that paired with one of the single strands, then another piece, and so on. That results in a duplex DNA with gaps that can be filled with the DNA polymerase. That was known; that's what Kornberg's DNA polymerase I does.

What Khorana did was to synthesize a gene by making single-stranded pieces that overlapped each other. And, whether they produced small gaps or large gaps, they could all be filled in. It was a very efficient way to synthesize a big piece of double-stranded DNA, just to make overlapping single-stranded pieces and use the enzymes to fill in the rest. You don't have to synthesize all of the stuff that the enzyme can fill in.

They used T4 ligase, because [at] every place where you fill in, you have ultimately to make a join. Now, there were two DNA ligases, *E. coli* DNA ligase and a similar enzyme that is encoded in a particular bacteriophage, T4. Now, *E. coli* ligase absolutely requires that there be a complete strand opposite the site to be joined for joining. But *E. coli* ligase cannot join two pieces of double-strand DNA at blunt ends. T4 ligase seems to be able to do both kinds of joins. But it does end-to-end joining inefficiently. In other words, it can join two blunt-ended pieces of DNA together. Sgaramella was in Khorana's lab, and my recollection is that he was involved in discovering that T4 ligase could do blunt-end joining.

Hughes: Did they have the idea that this was the possible mechanism for joining two foreign pieces of DNA?

Berg: Never heard anything about it. Sgaramella came to Stanford and was in the genetics department. He sat in on all our group

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[1] V. Sgaramella, J.H. Van de Sande, H.G. Khorana, *PNAS* 1970, 67:1468-1475.

meetings, so he knew all we were doing; he was a part of it. And he knew that T4 ligase could do this, and we knew that.

Hughes: But nobody was saying this was a possible mechanism for recombining DNA?

Berg: No. When Sgaramella came, I think Jackson was already here and involved in the recombinant DNA research. But, to my knowledge, Sgaramella had never either suggested or used T4 [ligase] to join two DNA molecules together before we did our experiments. In fact, I have the papers here. I've been carrying them around. I wanted at one point to write up something. I have the Sgaramella paper; it's 1972.[1] As far as I know, Sgaramella had never joined two DNA molecules together until he was at Stanford and while he was attending our group research meetings. We were working with SV40 DNA, and he said, "Well, I can join P22 with T4 [ligase]." Well, the joining efficiency of T4 ligase was extremely poor, whereas our joining was very efficient.

Hughes: So you wouldn't have considered using his system?

Berg: No, certainly not.

Hughes: It was never a discussion point when Sgaramella was sitting in on the meetings?

Berg: No; we were cooking away on this experiment, and we were not thinking of using Sgaramella's method.

The exact chronology of everything is not quite as clear in my mind as perhaps it should be. But Sgaramella was clearly in our group research meetings. He came frequently and was there during the time that Jackson was doing his work. I think there's no question that [Sgaramella] reckoned that instead of having to make cohesive ends, it might be possible for T4 ligase to join DNA molecules. And that idea grew out of the discussions and being part of a common group.

This is that little essay that I told you about; as far as I know it was written in 1969, '70, something like that.[2]

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[1] V. Sgaramella, "Enzymatic oligomerization of Bacteriophage P22 DNA and of linear Simian Virus 40 DNA," *PNAS* 1972, 69:3389-3393.

[2] Paul Berg, "Can oncogenic viruses be used to transduce cellular genes?" [n.d., Berg's personal archive.]

Hughes: Maybe by the content we can date it.

Berg: No, I looked through it again. I think I wrote it for a book, which I don't have a copy of here, a compendium on SV40, which was being published at Cold Spring Harbor. We were asked to contribute essays that had to do with SV40 for this book, and so I wrote this little thing, and then they eventually decided not to include the essays. The book became just a compendium of data about SV40. So, I had this in my file. [interruption for lunch]

A Method Difficult to Execute

Berg: Our work with recombinant DNA raised everybody's consciousness about making recombinant DNAs and putting them into organisms to do lots of new things. But, as I said, everybody realized that the method we used was cumbersome, technically challenging, and maybe not easily replicable.

Hughes: What about getting the enzymes that were necessary?

Berg: Yes. That's why I think everything happened at Stanford. I'm going to come to that next.

Discovery of Naturally Occurring Cohesive Ends

Janet Mertz

Berg: Janet Mertz was a graduate student in my lab. She was a very bright, energetic, ambitious kid. I think she graduated from MIT when she was sixteen, or something like that, with a dual degree in engineering and biology. In the beginning she was a pain in the butt, too.

I asked her to identify all the potentially infectible forms of SV40. SV40 is a covalently closed, completely contiguous, circular double-strand DNA. Two strands wind around each other, all the way around. I asked her to determine whether linear SV40 DNA was infectious. From bacteriophage, we knew that viruses with circular DNA genomes were totally noninfectious as linear molecules. She was then to separate the two circular strands and ask if the circular strands were themselves infectious and whether nicked circles were infectious. The latter are made by

introducing a nick in one strand; the product stays together as double strands, but there is a discontinuity on one strand. She started on this study.

Herbert Boyer and Restriction Enzymes

Berg: By that time, Herb Boyer up in San Francisco [UCSF] and some of his colleagues had been purifying restriction enzymes. One of the enzymes that Herb Boyer purified was called EcoR1. It came from an *E. coli* strain, and it was known to be part of one of the restriction modification systems.

Two years earlier, when I came back from La Jolla, I was curious about whether restriction enzymes could be used to cut SV40 DNA in a specific way. And so, I got a couple of restriction enzymes that were then known, isolated by [Matthew] Meselson at Harvard. One was called *E. coli* K, EcoK, and the other was EcoB. Francois Cuzin, a postdoc in the lab, tested them and found that they cleaved SV40 DNA, but at random sites. Instead of unique linear molecules with identical ends, both enzymes made a population of linears which had different ends. That was useless for what we wanted, so I forgot about it.

When Herb Boyer obtained EcoR1, I asked him if we could test it on SV40 to see whether it would make unique linears. And the answer was, it did. That led to a paper John Morrow published, in which he and I showed that EcoR1 made a unique single cut in SV40 DNA.[1] It cut the SV40 once, in only one place, and every linear was exactly the same. That was important because it gave us a reference point on the circle. The point of publishing the paper was, we could now relate all other sites on that DNA to this EcoR1 site.

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[1] J.F. Morrow and P. Berg, "Cleavage of Simian Virus 40 DNA at a unique site by a bacterial restriction enzyme," *PNAS* 1972, 69:3365-3369.

Mertz and Davis: EcoR1 Makes Cohesive Ends

Berg: In testing the infectivity of different forms of SV40 DNA, Janet also tested the R1 linears that John Morrow had made with R1. And, she found that they were infectious.[1]

Hughes: It surprised you?

Berg: Big surprise. Not as infectious as the circular DNA, but 5 or 10 percent as infectious. I said, "Janet, your linear DNAs are contaminated with circles. You didn't get complete cleavage." So, she did the experiment over, and over, and over again, purifying the linear molecules, but with the same result.

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Berg: So she began to look at them in the electron microscope, and sure enough, they were all linear. She didn't find any contaminating circles that could have accounted for the infectivity she found. Ron Davis, who was one of my colleagues in the department, was an expert in looking at DNA molecules by electron microscopy, but suggested that maybe they should look at the linears at lower temperatures. So he devised a way in which you could mount the DNA on the grid used for the microscopy, at low temperature. When they did that, many of the linears were circles.

Hughes: Did he have a rationale for that suggestion?

Berg: I don't know if he did or not. I don't remember that there was any reason for expecting that they might be circles at low temperature. I should say, he knew that was the behavior of these lambda DNA that had longer cohesive ends.

As soon as they found that, they realized that the ends were probably cohesive, but very short. And if they're very short, the stability of the helix that you can form is going to be dependent on the temperature. So, at room temperature, things are linear. Below 4 degrees Celsius they were circular. This was an astonishing finding.

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[1] J.E. Mertz and R.W. Davis, "Cleavage of DNA by R_1 restriction endonuclease generates cohesive ends," *PNAS* 1972, 69:3370-3374.

Boyer's Group: Sequencing the Cohesive Ends

Berg: Well, we called Herb Boyer and said, "Herb, we've got this astonishing thing. These R1-cut linears have cohesive ends." He came flying down here within the hour. [laughter] And we went through the data. We decided that we were going to characterize the EcoR1 generated ends, and he was going to sequence the ends. So Herb Boyer's paper that R1 made cohesive ends appears in the same issue with Janet Mertz and Ron Davis' paper, published back-to-back.[1] Both these papers appear in the same issue as the Morrow and Berg paper. Because *PNAS* did not allow an author's name to appear on more than one paper in an issue, my name does not accompany the Mertz and Davis names. They determine the sequence of the cohesive ends as being AATT.

First Experiment Using Cohesive Ends

Berg: Ron Davis and Janet Mertz performed the first in vitro recombination mediated by the cohesive ends created by this enzyme R1.[2] It is a fact that nobody acknowledges today.

Hughes: Why does nobody acknowledge them?

Berg: Because Stanley Cohen prefers not to. Stanley Cohen is given credit as being the first one to make recombinant DNAs, and he chooses to minimize the significance of that [Davis-Mertz] finding. Ron and Janet took two different DNAs, each with a different buoyant density, that is, when you centrifuge them, they separate because they have different densities. Both were cut with EcoR1, mixed and incubated with DNA ligase at low temperature, and then they were re-centrifuged. Most of the DNA now centrifuged at an intermediate density. That is, the two different DNAs had been covalently joined, giving molecules with an intermediate density. This was the first demonstration that you could use EcoR1 to do recombination in vitro.

The interesting thing is that issue of the *Proceedings of the National Academy of Sciences* has John Morrow showing that R1

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[1] J. Hedgpeth, H.M. Goodman, and H.W. Boyer, "The DNA nucleotide sequence restricted by the R1 endonuclease," *PNAS* 1972, 69:3448-3452.

[2] J.E. Mertz, R.W. Davis, "Cleavage of DNA by R_1 restriction endonuclease generates cohesive ends," *PNAS* 1972, 69:3370-3372.

makes a unique break in SV40, creating a reference point. The Mertz paper and the Boyer paper were in the same issue. The Sgaramella paper is also in that issue which is one or two issues later than the one in which the Jackson et al. paper appears.[1]

Berg: [interruption] I was just saying that once it was clear that this enzyme made ends which were cohesive and could, in fact, be used to join DNAs, it was obvious to most people now that you could join any DNA molecules together if they were each cut with EcoR. Take any two DNAs you want; cut them with EcoR1 to make sticky ends, and the two molecules can join together.

Peter Lobban's Contributions to Recombinant DNA

Thesis Proposal, November 1969

Hughes: Well, there are two other people that I think you should bring into this story. One is Peter Lobban. I have a copy of his dissertation, which is dated May, 1972. John Lear dates his proposal to his thesis committee to November, 1969.[2]

Berg: I might even have a copy of it here. Yes, there it is.[3]

So I came back from La Jolla in '68 with the idea to try to convert SV40 into a transducing DNA. Dave Jackson was a graduate student with one of my colleagues, Charlie Yanofsky, in the biology department. Dave wanted to come over and do a postdoc. He came over, I think, in late 1969, but he had to finish some experiments that he was doing for his Ph.D. thesis or a paper on

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[1] Berg noted the following: The Sgaramella paper was submitted to press on September 5, 1972 and appeared in the November issue of that year. The work in the paper was done while Sgaramella was at Stanford and attending our group meetings. The Jackson, Symons, Berg paper was submitted on July 31, 1972 and appeared in the October 1972 issue of *PNAS*. The Morrow-Berg paper was submitted August 16, 1972; the Mertz-Davis paper September 11, 1972. Both appeared in the same issue as the Sgaramella paper.

[2] John Lear, *Recombinant DNA: The Untold Story*, New York: Crown Publishers, 1978, p.43.

[3] Peter E. Lobban, "The Generation of Transducing Phage *In Vitro*," third exam, November 6, 1969.

his thesis work. So, he didn't get started on trying to join DNAs until sometime in early 1970, I would say.

Peter Lobban was a graduate student with Dale Kaiser. I was unaware that he had presented a proposal to recombine DNA in vitro by using terminal transferase to create synthetic cohesive ends. I wasn't on his proposition committee. Then when Dave [Jackson] began to start the experiments, we found out that Peter had actually proposed the same approach we had decided on. The concept of using cohesive ends came from Dale Kaiser, the same source from which I got it. Dale Kaiser was actively working on lambda cohesive ends, and Peter Lobban, in his research group, was clearly motivated by that, as was I. Normally, these Ph.D. propositions are exercises in which people present an idea. They're supposed to demonstrate a capacity for being creative in generating a new idea, devising an experimental way of testing their idea, and then they continue on their thesis, whatever that is. Most often, the proposal and thesis are totally unrelated.

Lobban's Communication with the Berg Group

Berg: I guess Peter's committee, or Peter himself and Dale Kaiser, were so struck by the novelty of the idea that Peter dropped what he was doing on his thesis, and he started to try to do what he described. My recollection is that in the beginning, unbeknownst to me, when Dave Jackson started on it, as is often the case, the people who were at the bench talked more to each other than I talked to Peter Lobban. And Dave found out that Peter Lobban was also trying to develop the same technique. The two of them, if they didn't actually collaborate, were in very close communication. What was very interesting was how easy the communication was.

Lobban's Discoveries and Speculations for Practical Application

Berg: Peter, and we actually refer to him in our paper, made several critical discoveries that facilitated the technique. For example, when you cut DNA and both ends are either flush or very close to being flush, it's very difficult for terminal transferase to add nucleotides onto the end. On the other hand, if you peel back the 5-prime strand, and you have the 3-prime end sticking out, then this enzyme adds nucleotides very well. Peter discovered that.

He very quickly communicated that to us, so that if we were going to make cohesive ends, it was better to pre-treat the DNA with an enzyme that cuts back the 5-prime end a little bit; we acknowledged Peter's contribution in our paper.

And so, there was a very close communication, but it wasn't a collaboration. We never intended to be working on the same project. Since I'd never seen his proposition, I was unaware of the implications of what he foresaw for being able to do this. Our research was strictly motivated by trying to get DNAs to attach to SV40 so we could introduce them to mammalian cells.

Hughes: What does Lobban speculate?

Berg: I think he begins to see this as gene therapy. It has been a long time since I've read his proposal. [Reading from a copy supplied by Hughes]: "An eventual goal for the method, assuming its success, a simple task would be to produce a collection of transductants synthesizing the products of genes of higher organisms." In other words, he would attach something that would get it into *E. coli*. "This would be of immense help in the purification of proteins made by these genes, for in many cases they would be far less dilute in the bacterium than in the cells of their origin." So he begins to see that it's possible to manufacture the products of genes, which ultimately came on much later.

"It is even conceivable that transduction can be used for fine structure mapping of the genes they bear. For phage can be mutagenized, and a collection of point mutants could be obtained." He was already visualizing that you could begin to make mutations in whatever you made. And, if you put it back into cells, you could determine whether the mutations affected the function, and so on.

Hughes: In the dissertation, he mentions the possible uses of this technology, and, of course, the main one is for transduction. He talks about the DNAs being used "in a search for proteins and other macromolecules present in mammalian cells that might interact with DNA in a gene-specific manner and thus be involved in control of gene expression."[1] And he talks about fine structure mapping. "[The method] could be used as a source of the

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[1] Peter Edward Lobban, "An enzymatic method for end-to-end joining of DNA molecules," Dissertation, May 1972. (Lane Medical Library, Stanford, dissertation #3781 1972L)

gene products that might be far more convenient than the mammalian cells themselves."[1]

Berg: Yes, that's what's in this proposal.

Not Cloning

Hughes: So, Lobban has the idea of cloning.

Berg: Well, he doesn't have the idea of cloning. I think what he has the idea of is constructing molecules which could then be introduced into cells that would express the transduced segment.

The Cohen-Boyer DNA Cloning Experiments

Berg: But the cloning really was a very different procedure. As far as I remember, the first real notion of cloning comes out of the experiment that John Morrow did together with Herb Boyer and Stanley Cohen where they cloned the ribosomal DNA sequences from the frog.[2]

Hughes: The very first cloning experiment was published in 1973, using--

Berg: pSC101.[3] Stan joined it to another plasmid.

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[1] Ibid.

[2] J.F. Morrow, S.N. Cohen, A.C.Y. Chang, H.W. Boyer, H.M. Goodman, R.B. Helling, "Replication and transcription of eukaryotic DNA in *Escherichia coli*," *Proceedings of the National Academy of Sciences* 1974, 71:1743-1747.

[3] S.N. Cohen, A.C.Y. Chang, H.W. Boyer, R.B. Helling, "Construction of biologically functional bacterial plasmids *in vitro*," *Proceedings of the National Academy of Sciences* 1973, 70:3240-3244.

Hughes: There is a paper, which precedes the *Xenopus* [frog] paper, in which Cohen speculates on the practical possibilities of this procedure.[1]

Berg: My recollection is that the introduction of a foreign, totally unrelated DNA, frog DNA, into a bacterial plasmid, introducing it into bacteria and getting the bacteria to propagate these was the first demonstration that you could propagate foreign DNA in bacteria. And two, that each colony produced from this was a clone, a clone of the original DNA.

In fact, the Morrow experiment starts with a piece of DNA which is a mixture, not a unique segment of DNA, and therefore each colony represents only one component of that mixture. That's cloning. Now, what I remember that Stan Cohen did was to take a *Staphylococcus aureus* plasmid, fuse it to the *E. coli* plasmid, and show that it can introduce the drug resistance property of the *Staph aureus* plasmid to *E. coli*.

Hughes: But, there's no talk about cloning?

Berg: I'd have to go back and read the paper in detail to know. I believe that the big breakthrough was the Morrow experiment, showing that you can actually clone foreign DNAs. That's my recollection.

More on Recombinant DNA

More on Lobban-Berg Group Interactions

Berg: Now, to go back to Peter Lobban. Peter and we decided that we should try to publish together. Then, at one point, Peter said, "No, I don't want to publish in the *PNAS* [*Proceedings of the National Academy of Sciences*], I want to publish it in *JMB* [*Journal of Molecular Biology*]. And, I would rather wait."

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[1] A.C.Y. Chang and S.N. Cohen, "Genome construction between bacterial species *in vitro*: Replication and expression of *Staphylococcus* plasmid genes in *Escherichia coli*," *Proceedings of the National Academy of Sciences* 1974, 71:1030-1034.

Hughes: I've read a slightly different account.[1] Dale Kaiser wasn't quite satisfied and wanted Peter to do more research.

Berg: Could be; I don't remember. They knew we were going to publish. There was never any question that we sneaked in a publication; they knew we were going to publish it in *PNAS* and when.

Hughes: And you each cite each other. I noticed that as well.

Berg: Oh, yes. It was kind of a friendly and collaborative program, with the understanding that we were each trying to do something different, but using the same idea. I believe we hit on this idea independently--I have no doubt about that--because I was totally unaware of Peter's having presented his proposal.

We had a visit from Jim Wang from Harvard. He reported that he found two circular DNA molecules called concatamers intertwined in certain cells. And, when I heard his seminar, which would have been probably late '68, early '69, I thought maybe that was the way we could get DNA into mammalian cells. That is, we would use SV40, which is circular, and we would try to take foreign DNA and circularize it with SV40 to form concatamers. If the SV40 DNA went into cells, it would carry with it this foreign DNA. Well, we tried different ways to generate such concatamers, and we failed. So, then we decided, well, maybe another way to do it is to open up the SV40 ring and join DNA segments to it by making cohesive ends. And so that's sort of the genesis of our idea.

It wasn't so novel because we knew that cohesive ends worked. And we knew that there was a way to make cohesive ends. And so that's what I laid out for Dave Jackson as a postdoc project. But he had to finish up research in progress in Yanofsky's lab, and he was delayed in getting started. Once he got started, he discovered that Peter Lobban had come up with the same idea and was working on it. And then, of course, we realized that we were both trying to do the same things, but for different purposes. We had a specific purpose in mind. Peter was more interested in a general method for joining DNA molecules. We were less interested in the way to join DNA molecules as making molecules that we could use.

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[1] Peter Lobban to Arthur Kornberg, October 10, 1986 (Kornberg's personal correspondence).

Berg Questions Lobban's Use of Two Identical Molecules

Hughes: Isn't your different goal indicated by what you chose as your system. Namely, you were using SV40 and lambda dv gal.

Berg: I don't remember what Peter used.

Hughes: He used two p22 molecules.

Berg: Yes, that's right. My own view was that, if you want to develop a method to do something, you'd want to do it with things that you could then do the next step. What was the endpoint of joining two p22 molecules together?

Hughes: Well, I thought about that, too. I thought, well, here's a graduate student trying to pick a circumscribed problem. He's just worried, can he join two molecules? What the molecules are doesn't matter to him.

Berg: Yes, but he writes and projects multiple uses and potential.

Hughes: Well, I know he does. I guess you could still say, why not use two different molecules?

Berg: Why not try it out with the molecules you ultimately would like to see some function for?

Hughes: Maybe there's something in the dissertation about something physically favorable about using two p22 molecules. I didn't copy the whole dissertation.

Berg: [scans dissertation] Yes, see: He refers to Sgaramella here--"Sgaramella personal communication, May '72." "So far the enzyme"--T4 ligase--"has been used to join certain small synthetic DNA molecules and to make linear dimers and trimers of P22 DNA...but no circles. The advantage of the ligase-mediated joining reaction is it's simplicity, for whenever the molecules to be joined have base-paired ends, only a single reaction is required. However, there is no way to ensure that a given DNA molecule will not join with another molecule of the same type..." Whereas, when you make cohesive ends, you direct who's going to join with who.

Hughes: Yes, exactly. But we haven't answered why he chose p22.

Berg: I don't know. I thought at the time--I don't know that I thought very deeply about it--but I couldn't understand why he was spending time with p22, anymore than why Sgaramella used p22. We

wanted to develop a joining method to actually construct something that we could test. That may not sound very profound--

Hughes: I understand what you're getting at.

Berg: In fact, today, if someone develops a technique or a method and tries to publish it, we ask, "What question are you trying to answer?" It would have to be some dramatic new technology that's going to open up a whole new world before a journal will actually publish something on a technique, and even then it's hard [to get it published]. But, if you do it in the context of answering a question, you develop a way to test or answer a new question, then the journals are more willing to accept the paper.

The Jackson, Symons, Berg Paper, October 1972

Berg: That's what we thought we were doing, and that's what we outlined in our paper. We certainly visualized some of the things that could be done with that approach. I can't remember whether our paper actually suggests growing SV40 in *E. coli*. That paper exceeded the length limits of the *PNAS* by one page. They in fact accepted it, for very special reasons, they said. But, it allowed us to discuss the implications.

Hughes: What were the special reasons?

Berg: They recognized that this was a breakthrough. In fact, I think there was an editorial in *Nature* about it. So, it was clear that right away the world saw that the ability to join DNA molecules together in vitro opened up a whole new direction.

Lobban's Work Ignored

Berg: And I think much to the dismay--I shouldn't say dismay, but certainly it was unfortunate--Peter Lobban's work got very little attention. To this day, a lot of people look back and don't understand why, except if you know Peter Lobban. He's very, very low-key, very phlegmatic. He went off to Canada, did a postdoc in a very different area, cell biology. Then he began to interview for jobs, and [spoken with pause between each word:] did - not - get - any - offers - for - jobs. He's a remarkably bright guy. He gave up and got a job and went back to engineering school. And I think, for people who knew Peter, that's his bent. His bent is

inventing new things and applying them. He now works for a company of which I'm a director.

Hughes: Small world.

Berg: He works on the engineering aspect of this company's project.

Hughes: I'll look up the date you submitted the Jackson, Symons, Berg paper. [July 31, 1972]

You wrote a letter to Hilary Koprowski in May of 1973 in which you mentioned your relationship with Peter:[1] "...at all times we had a very amicable, cooperative and most important complementing "collaboration", so much so, that it was Peter who often made the key breakthroughs to solving technical problems." That's an almost contemporary account of what you had already told me.

Berg: Yes, almost the same words.

Recombinant DNA: Jensen, Wodzinski, and Rogoff, 1971

Hughes: In his dissertation, Lobban mentions a paper by Jensen and colleagues.[2]

Berg: I'd never heard of him. Stan Cohen wrote me a letter after that Philadelphia meeting in which he tells me about Jensen. He might have mentioned it in his talk at the Beckman Center History Library Symposium. But I frankly had never heard of Jensen before.

Hughes: Well, Lobban knew about him. Here it is. [Indicates page in Lobban dissertation]. Read it aloud, if you don't mind, for the tape.

Berg: Continuing from Lobban's thesis, "During the course of these experiments, a paper appeared in the literature (Jensen, Wodzinski, and Rogoff, 1971) describing an attempt to join

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[1] Berg to Koprowski, May 22,1973 (Berg papers, SC358, Green Library, Stanford, box 3, folder:1973).

[2] R.H. Jensen, R.J. Wodzinski, M.H. Rogoff, "Enzymatic addition of cohesive ends to T7 DNA," *Biochemical and Biophysical Research Communications* 1971, 43, no.2:384-392.

molecules of the DNA of T7 phage together by a method that called for the use of terminal transferase to create cohesive ends. The conclusions reached by the authors were much more pessimistic than those stated here. They made what, in our symbols, would be dA-T7-dA and dT-T7-dT, but when the two DNA's were annealed, joining was relatively poor, and no circles were seen even though linear dimers and trimers were formed. It would appear, then, that in their hands terminal transferase did not add homopolymer blocks to both ends of very many of the molecules; the reason is that they did not treat the T7 DNA with lambda exonuclease prior to using the transferase." That's in order to peel back the end.

Hughes: I see.

Berg: [continuing to read from Lobban's thesis] "In our hands, T7 DNA primes the transferase reaction with the same kinetics as P22 DNA; that is, there is an acceleration phase. The authors also were unable to close their joined molecules with ligase alone or with ligase with polymerase; the reasons for that problem are not obvious."[1]

I don't remember ever seeing that paper by Jensen or hearing anybody even discuss it. I can't believe it's blocked out [of my memory] completely, but I don't remember ever hearing about it. In 1971, we were well along on the work. If Peter knew about [the Jensen et al. paper], it would be surprising that Dave Jackson would not have known about it. But certainly, I don't remember ever hearing anything about it.

Recombinant DNA Construction Using Terminal Transferase

Berg: Well, actually, there was another person who was trying to do the same thing whom I knew but didn't know about his work. I went to give a seminar at Merck where I described this recombinant work. This guy, who was working at Merck [and] had been a student of one of my friends, told me that he had come up with this same idea of constructing recombinants using terminal transferase. The people in power at Merck who decide on what people work on said, okay, he could give it a try. He worked on it; he couldn't get it to work for about four, five, six months or something, and they ordered him to give up the project.

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[1] Lobban dissertation, May 1972, pp.120-121.

It was only several months later that I came to give the seminar and described the successful use of that approach. He was not devastated but he was certainly, as you might expect, kicking somebody for having prevented him from working on it longer. The surprising thing is it was actually easy, but he did not have access to the same enzymes, although he had worked in a DNA replication lab. He had gotten his degree with Jerry Hurwitz, so he knew how to use enzymes. I'm blanking on his name. Fred something.[1]

David Hogness: Cloning of Eukaryotic DNA

Hughes: I read that David Hogness very early began to use the technique with eukaryotic DNA.

Berg: Dave used the technique of dAT joining. My recollection is that he did it with plasmids. He wanted to clone *Drosophila* DNA. He wanted to isolate segments of the *Drosophila* genome. If you cut DNA with EcoR1, you can only cut it in defined locations, at sequences that the enzyme recognizes. But, if you want to make random breaks so you get all possible sequences, then you make other kinds of breaks. You shear the DNA. If you run it through a Waring blender, the forces cause the DNA to break, and they break at random sites. Now, if you do that, you don't have cohesive ends. So then you have to build the cohesive ends.

But, this was after Morrow, Cohen, and Boyer showed that you could actually clone a foreign piece of DNA. So Hogness said, "Okay, I'm going to use a plasmid. I'm going to make AT ends on the plasmids, and I'm going to take *Drosophila* DNA, shear it down to a distribution of sizes. I'm going to put A's or T's on it, and then I'm going to clone it, which is what he did.

And so, he was able to clone, for the first time, bits of *Drosophila* DNA in bacteria. I don't think many people outside of Stanford used that approach. Well, I'm not sure. There could have been some other people who used AT joining. But not too many people used AT joining because they just thought that it was cumbersome, difficult.

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[1] In editing, Berg could not recall the scientist's last name.

Discovery of Other Restriction Enzymes Making Cohesive Ends

Berg: And then, it was discovered that other restriction enzymes also made cohesive ends. We began to develop a battery of ways to cut DNA leaving cohesive ends. If you use BemHl, another enzyme, it cuts DNA but creates different cohesive ends. If you cut the plasmid with the same enzyme, then you can join those together. As the number of restriction enzymes began to increase very quickly, we learned which ones produced cohesive ends, which ones didn't. So now you have a whole battery of tools to be able to join different DNAs together. You want to restrict it by saying, "I'm only going to cut the DNA at Rl sites."

Stanley N. Cohen and the Cloning Experiments

Departmental Affiliation and Early Research Interest

Hughes: You had a relationship with Herb Boyer which predated his recombinant DNA experiments. And was Stan Cohen--?

Berg: Stan Cohen was in the Department of Medicine.

Hughes: Not in Genetics at that point?[1]

Berg: He was in the Department of Medicine. I think he had a joint appointment. But, he was primarily the head of the Division of Clinical Pharmacology in the Department of Medicine. Because I was very close friends with Jerry Hurwitz with whom Stan was a postdoc, I knew all about Stan Cohen. When he came to Stanford, he decided to work on plasmids because plasmids were important in clinical medicine. It was clear that plasmids were the carriers of drug-resistance genes, and drug-resistance was a big problem in clinical medicine, and so he was studying the carriers of drug-resistance. Stan did a very nice job of characterizing the circular DNA molecules. If you ask where does the person who has those kinds of interests find their intellectual mates, it is in biochemistry. And so, he hung around in [the Department of] Biochemistry most of the time.

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[1] Cohen joined the Department of Genetics in 1978.

Mort Mandel's Procedure for Introducing DNA into Bacteria

Berg: When he was isolating plasmids, one of the experiments he wanted to do was to get plasmids back into bacteria. And there was a postdoc, or sabbatical visitor, in Dale Kaiser's lab. Mort--

Hughes: Mandel?

Berg: Mandel had discovered that if you took *E. coli* and exposed them to elevated calcium and gave them a shock, DNA entered these cells much, much more frequently and efficiently than if you didn't do that. And so, when Stan heard that, he came around and wanted to learn how to do this. Now, Janet Mertz, who was in my lab, was already doing that. And so she instructed Stan on how to do this, and they actually worked together.

Berg's View of the Genesis of the Cohen-Boyer Experiments

Berg: When the R1 experiment showed that the existence of cohesive ends could be used to join DNAs, Stan immediately grasped the relevance of that as something he could do. And he said, "Okay, if that's true, I can take two different plasmids, cut them with R1, anneal them together, ligate them, and then see if I can put them back into cells. Janet was helping him with the technology of reintroducing DNA into cells.

We were aware of those experiments. Herb Boyer was not involved at all as far as I know. Then the story goes that Cohen and Boyer were at a meeting in Hawaii, and they were sharing pastrami sandwiches, and they had this great idea that maybe they could in fact reconstruct new kinds of plasmids and put them back into bacterial cells. If I remember, Stan had just taken pSC101, cut it, and shown that you could make dimers. But now the discussion with Boyer was to do this experiment of joining two different DNAs together, and they did that. I'm not sure who in Stan Cohen's lab did that experiment.

John Morrow and the *Xenopus* Experiment

Berg: At the same time, John Morrow was finishing up his Ph.D. in my lab. And he was going to do a postdoc with Don Brown at Carnegie. Brown had sent him some frog ribosomal DNA, for what reason I

don't know. But it was in Morrow's refrigerator. Unbeknownst to me, and without him telling me, Morrow went to Stan Cohen and Boyer and said, "We ought to be able to introduce this frog DNA into *E. coli* by linking it to the plasmid."

Hughes: You told me that this was ribosomal DNA. They were using this particular DNA because--

Berg: It was foreign. John happened to have it in his refrigerator. I suppose you could have asked, if I put it into bacteria, would this frog ribosomal DNA be expressed? And maybe that was on his mind. But, John never told me anything about this. He kept telling me that he was delayed in finishing his thesis because of computer problems, or this problem, or that thing, all of which was beginning to be very fishy.

Hughes: Were you suspicious at the time?

Berg: I wasn't suspicious at the time. I had no idea. People in the lab knew, but nobody said anything to me about it. Eventually, the experiment was done, and John came to me and told me about it. I almost kicked him out of the lab, I was so furious. He was using me and lying to me about what was delaying his departure. In fact, surreptitiously, he had gone off-- He had every right to do that, but at least he could have been upfront about it.

Well, years later, I've gotten many letters from John Morrow reminiscing about that period and how remorseful he had been and how much he credits me with not having destroyed his career. The fact is, our department went off on its retreat. He remained behind because he was literally finished, and people who are finishing up their thesis usually don't come to our retreats. But because I thought the experiment was so terrific, I called him and invited him to come and give a talk to the department on this experiment. He drove down to Asilomar and gave the talk. And he has acknowledged that. The point is, I did not know about that experiment when it was being conducted. I regard it as one of the critical experiments in the whole evolution of the DNA cloning technology. Stan and Herb either ignore it or fail to give it sufficient credit.

Morrow and Helling Challenge Patent Inventorship

Berg: Regarding the Cohen-Boyer cloning patent, John Morrow hired lawyers and sued them because they excluded him from the patent,

which essentially was based on cloning. And Helling also sued them.

Hughes: But they remain off the patent.

Berg: Yes, that's right. They were told that patent law says it can't have more than two people as inventors; I don't even know if that's true [it's not].[1] But, in any case, John was resentful for a long, long period afterwards. He was screwed out of any of the benefits from that or the recognition of it.

Berg Claims Two Key Experiments in Cloning DNA

Berg: So, I regard that two key experiments were done in our department; they were both done by my students, but not necessarily under my supervision: Janet clearly discovered the cohesive ends of R1--she and Ron Davis--and showed for the first time that they could be used to join DNA molecules together. And John Morrow was the first one to clone defined DNA sequence in *E. coli* and by that experiment establish the concept of cloning. And neither one of them is widely acknowledged. People who know recognize what they did. But they never have gotten the kind of credit that Boyer and Cohen got. Well, once the experiment was done with *Xenopus* DNA, it became pretty clear that you could put any DNA into *E. coli*. And, that's when the whole thing exploded.

The Commercial Potential of Cloning Technology

Berg Doesn't Hear of It

Hughes: Were you thinking about the commercial potential of this technology, before the *Xenopus* experiment?

Berg: Not me; I never heard anybody talk about it. That's why I would say it's sort of a surprise to see it in Peter's proposal, because I had never seen this proposal. I'd never heard anybody talk about potential commercial value.

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[1] For the history of this major patent, see: Sally Smith Hughes, "The Cohen-Boyer Recombinant DNA Cloning Patent and the Accelerating Commercialization of Academic Biology" (in press).

Genentech and Cetus Palo Alto

Berg: The first I heard of commercial discussions was that Genentech was being formed, largely through Herb Boyer. I think in those days most of us who were academics were somewhat disdainful of commercial involvement. In fact, it created a lot of problems with UCSF, enormous angst: Who did what? And who owned what? And so on and so forth. There were probably suits between UCSF and Genentech about materials. Genentech didn't have a place to work. So Herb Boyer, who was "Genentech", was working at UCSF laboratories, and that, most of us thought, was totally improper. And then, not long after, Cetus actually formed a laboratory for Stan Cohen. It was called Cetus Palo Alto, but after some time it failed.

Hughes: Why did it fail?

Berg: Can we turn that recorder off? [interruption]

Berg's Nobel Prize Address: Citation of Cohen-Boyer Research

Hughes: I read your Nobel address, in which you cite a lot of work, but you don't cite the Cohen and Boyer papers.[1]

Berg: My recollection is that the Nobel address was largely focused on the work that we were doing with SV40 mapping and so on. It had nothing to do with cloning or recombinant DNA. It was done in 1980. It describes the dissection of SV40. I think it's called "The Dissection and Reconstructions of Viral Genomes". It has to do with identifying the locations of the genes of SV40.

Hughes: I have the paper. [pause while Berg scans paper]

Berg: You have to find out what references 29 and 30 are here. Because I say, "Since that time there has been an explosive growth in the application of recombinant DNA methods for a number of novel purposes and challenging problems. This impressive progress owes much of its impetus to the growing sophistication about the properties and use of restriction endonucleases, the development

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[1]Paul Berg, "Dissections and reconstructions of genes and chromosomes," Nobel lecture, December 8, 1980, *Bioscience Reports* 1981, 1:269-287.

of easier ways of recombining different DNA molecules, and, most importantly, the availability of plasmids and phages that made it possible to propagate and amplify recombinant DNAs in a variety of microbial hosts (see references 29 and 30 for a collection of notable examples.)"

If you go back there, my guess is that you might very well find that these are references to either--

Hughes: I'll get them for next time.[1]

Berg: I have never failed to acknowledge Cohen and Boyer. I can give you copies of many lectures and talks; I always acknowledge Cohen and Boyer for being the key people in developing cloning technology. And there's no question that we did not clone anything. I have said that many times. The confusion lies in whether we were aiming to clone things and didn't understand the system well enough to know that we couldn't have cloned things the way we did it. But, in point of fact, as I've told you, our aim was to get things into mammalian cells, not to clone things in bacteria. That was something that somebody raised as a possibility from the point of view of risk, which was never in our plans.

More on Berg's SV40 Experiment: No Expectation of Cloning

Hughes: Well, another issue was raised: if you had known that the way the SV40 DNA was inserted would prevent replication, would you have recommended the moratorium?

Berg: What do you mean by if we had known?

Hughes: Well, from what I understand of the science, the way the DNA was actually inserted interfered with a gene that was required for replication.

Berg: Oh, okay. The piece of bacterial DNA we used is actually a plasmid. It has a replication origin and the genes needed for replication, and it has these three bacterial genes. We opened this piece of DNA in a region of the sequence which is necessary

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[1] References 29 and 30 refer to issues of *Science* devoted to articles on recombinant DNA; there are no papers by Cohen and/or Boyer. (*Science* 1977, 196, no. 4286, April 8, 1977; 1980, 209, no. 4463, September 19, 1980.)

for it to replicate. And so, when we stuck the SV40 into it, this molecule would not have replicated in *E. coli*. We didn't know that. But since that wasn't our objective, it would not have even occurred to us to know whether this was going to replicate in *E. coli*.

Actually, the way to make it replicate in *E. coli* would have been to make a dimer. So had that been our goal, we would have solved that issue. But, that wasn't our goal. We weren't thinking along those lines. Our goal was to open SV40 in a region which left the SV40 genes intact. We could have used random opening of SV40 because some of the molecules would have been open in a region which did not inactivate any genes.

It turns out that R1 broke right into one of the SV40 genes. And while we could have made a recombinant, no problem, by R1, it would not have been able to express the major capsid protein. Stan has raised the point, "Aha, you would never have been able to clone it." And I said to him, "Stan, that was never our intention, that wasn't the goal of the experiment. The goal of the experiment was to create a transduction system for mammalian cells. We weren't looking to put things into *E. coli*." I acknowledge that freely. And, therefore, our experiments didn't open the door to cloning in bacteria because that wasn't what we had in mind.

All that we did was raise the consciousness that you could join DNA molecules together outside a cell. And, once you begin to think about what was necessary to get recombinant DNAs to do certain things, people began being more sophisticated about what gets joined to what and where it gets joined.

More on the Biohazards Controversy

Berg: The Morrow experiment, if I remember correctly, would have been early '74.

Hughes: And the first Cohen-Boyer paper was published in November 1973.

Berg: [June] '73 was the Gordon conference, because Boyer reported the first cloning experiment which got a lot of people uptight. And '74 was the Morrow experiment. The '73 experiment reported at the Gordon conference got people excited and triggered the National Academy of Sciences to get involved. It got me involved.

MIT Meeting to Discuss Biohazards, 1974

Berg: As we were readying the meeting at MIT to discuss how to advise the Academy, up comes the Morrow et al. paper. The Morrow paper shows that anything can be inserted into a plasmid and be cloned. That raised the stakes. Stan Cohen said, "I'm getting telephone calls from all over the world, people asking me to send them pSC101. What should I do?" So when I went to MIT for the meeting, it was with the background that it was now clear anything could be put into *E. coli*.

Some of the things that Stan Cohen said people were calling him about sounded ominous! Should the tetanus toxin gene be put into *E. coli* and enable *E. coli* to do something it can't ordinarily do? So, those were the things that led to our consideration of a moratorium. Interestingly enough, Stan was not involved in that committee meeting at MIT, nor was Boyer, nor was Hogness, nor was Davis. But, as soon as we brought back the outline of what we had decided on at that meeting, they immediately wanted to have their names associated with it. That's how they became signatories of the "Berg" letter.

Hughes: Am I remembering correctly that you were the organizer of that MIT meeting?

Berg: Yes.

Hughes: Had you invited them?

Berg: No. I had invited primarily people who had been involved in Asilomar I, who had some experience in thinking about the risks of this kind of experimentation. Jim Watson, because he had been outraged by some of the things that people wanted to do with viruses. Dave Baltimore, because he clearly had been one of the principals in working with animal viruses, particularly tumor viruses. So, all the people who came to that meeting were people who had been involved in that first Asilomar meeting.

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Berg: I also invited Maxine Singer but she couldn't come. I don't remember if we invited anybody else that couldn't come. But we felt that seven was a reasonable size. Remember, we were not on a mission to do what we did. [laughter] I had been asked by the president of the National Academy of Sciences to advise the academy on how to respond to the concerns that had been voiced at the Gordon conference. It was a very limited request. I just took it on myself to say that I wasn't going to advise the

president of the academy on my own. And what I would do is try to bring some people together that I thought would be thoughtful and do something about it and just asked these seven or eight people. It turned out there were six others beside myself.

The "Berg" Letter, July 26, 1974[1]

Berg: But when I came back to Stanford, I reported that the group's decision was to publish a letter in *Science* and *Nature*, calling attention to the potential biohazards and offering recommendations on how to proceed. Stan, and Herb, and Ron Davis, and Dave Hogness immediately wanted to have their names associated with this.

Hughes: Why?

Berg: I don't know. Stan Cohen and Dave Hogness later thought the whole thing was stupid. Nevertheless, they signed the letter, as did Jim Watson. At the [Asilomar II] meeting, Jim Watson was most vociferous about calling for a moratorium. Why they did it? I don't know. Maybe they thought, as we did, that there was enough uncertainty that it was a reasonable thing to do. Maybe they did it because they wanted to have their name associated with something that would have that flavor. I just don't know. But, we did not exclude them. There was never a question of voting, should we or should we not. These people wanted to put their name to it.

My recollection is that I probably thought it was a good thing, because they were active participates, their names were very well-connected with recombinant DNA already. Therefore, if their names were on it, this [letter] would have more of an impact on the rest of the community.

Hughes: Did they come to you and ask to be put on the letter?

Berg: Yes. Well, I'm sure I circulated a draft of what we had come up with. And they said, could they put their names on it too?

Hughes: I can see that it would work to support the letter's recommendations to have people sign this letter who were actually involved in these experiments.

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[1] Paul Berg and ten signatories, "Potential biohazards of recombinant DNA molecules," *Science* 1974, 185:303 (July 26, 1974).

Berg: I think that was in the equation. There was never any thought that they should be excluded. And so, I don't think there was a big argument, for example, pros and cons. It seems not an unnatural thing and might very well have been obvious that if you've got the very people who were most involved in the work, actual participants of the work, the letter would have more force.

Don't forget, the letter was addressed to colleagues around the world; it wasn't addressed to the local community. The point is, if you had names like Cohen, and Boyer, and Hogness, and Davis, in addition to the other people that had been on it, the letter would have more impact, more acceptance.

The Nobel Prize in Chemistry, 1980

Singling Out Berg

Hughes: Let's go on to a discussion of the Nobel Prize, even though it's a few years forward in time. I read the article in the November 1980 of *Science* which quotes the press release from the Swedish Royal Academy: "Berg was the first investigator to construct a recombinant DNA molecule, i.e., a molecule containing parts of DNA from different species."[1] Do you think that phraseology is meant to give the reader a fully descriptive idea of what the research was about? Or was it a way of tacitly explaining, to those who really understood the body of research, why you had been singled out rather than someone else, namely that you were the only one to have joined DNA from different sources?

Berg: I don't know who is the author of that statement; the actual statement for the Nobel Prize talks about my early work as well as the recombinant DNA. Not as explicitly as that. Essentially, we worked in nucleic acids and recombinant DNA. I don't even know where the statement you quoted comes from.

If you talk about recombinant DNA, you talk about joining two different kinds of DNA molecules together, and our experiment was clearly the first to do that. What Peter Lobban did was make dimers. What Sgaramella did was make dimers. And whoever this other guy was?

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[1] G.B. Kolata, "The 1980 Nobel Prize in Chemistry," *Science* 1980, 210:887-889.

Hughes: Jensen.

Berg: Jensen made dimers. Is that a distinction? I don't know. I disdain the kind of identification--"the father of genetic engineering", all that kind of hype that comes around. I have never claimed that we developed cloning; we didn't. What we did was develop a way to join two different DNA molecules together outside of a living cell--period.

Berg's Opinion of His Best Work

Hughes: If you were the Nobel committee and could choose amongst your own scientific achievements, would you choose the recombinant DNA work?

Berg: You mean, would I have chosen that bit of work for me to get the prize?

Hughes: What I'm really asking is, do you consider it to be your best work?

Berg: No, no, no, not at all. But, you see, that's not the criterion that the Nobel committee uses. The man who shared the prize with Kornberg, [Severo] Ochoa, got the prize for--presumably--having discovered the means for making RNA. But in fact we know that the enzyme he discovered doesn't make RNA at all. It's like that terminal transferase; it's a dumb polymerase that just polymerizes nucleotides at random. But Ochoa was one of the towering figures in biochemistry. Everybody would have said that this guy was going to get the Nobel Prize. What did he get it for? He got it for something which he would not have identified as his most notable achievement. I think you can say that for any number of Nobel awards.

I might have told you very early in our interview process that I thought the most significant and most innovative piece of work that I did was as a postdoc when I joined Kornberg's lab, in the discovery of this new class of compounds for activating molecules for assembly into larger molecules. I think that was much more creative.

Speculations on Why Berg Was Chosen

Berg: But remember, recombinant DNA had two things going for it. One was it was at the focus of a public policy debate all over the world, in Sweden as well. And two, it had an enormous impact. What the Nobel Prize often rewards is the way science is changed as a consequence of that discovery.

Carl Cori was one of the great figures of biochemistry in the 1940s. He and his wife Gerty shared the Nobel Prize for discovering an enzyme that breaks down glycogen. If you ask any student today who Carl Cori was, they wouldn't know. And two, if you asked them if they thought that glycogen phosphorylase had a major impact or changed the face of biochemistry, they would answer no. But, in its day, it was an innovative finding. Remember, they had already established themselves as premiere biochemists.

So, I'd like to think that the Nobel Committee also considered the highly regarded work that I had done long before the recombinant DNA. Perhaps some people were supportive because I was a leader in the public policy part of it. And in some circles that seems to be more important than the scientific part.

Hughes: Really?

Berg: Yes. I don't know whether the people on the Nobel committee used that. More likely, however, is the enormous impact and the way [recombinant DNA technology] changed the way biology is done. Anybody, looking back, could ask how this enormous new kind of science began. Where does it go back to? It starts with the demonstration that you can join DNA molecules together. And then a lot follows from that.

I was invited shortly after the public policy issue broke--publication of the moratorium letter--to come and give a lecture in Sweden, which the king attended. It was a big public lecture. Swedish opinion was much more interested in the issues of responsibility in science, and ethics, and safety. And so, I could have become a hero, I don't know. There might have been some people that would have regarded what I did as being courageous. I don't know. I didn't think it was courageous.

But, people look at that whole episode in different ways. It has really been amazing. We focused on it very narrowly: is [recombinant DNA research] safe, is it not safe? People saw it as raising a big ethical debate and us displaying an enormous level of ethical concerns. But we saw it as a public health issue.

Hughes: Well, you have been criticized for narrowing the debate to the issue of biohazards.

Berg: That's right. And, I think that diminishes any attribution of high ethical standard. If you said we were in this to really raise the consciousness of the world about the impending change and the way things were going to be done, we didn't do that!

Hughes: I think associated in the public mind is the idea of scientists policing themselves.

Berg: Yes.

Hughes: That idea could have influenced the Nobel committee.

Berg: After the award was announced, there was an editorial in *Nature*, or a little blurb about the award. My recollection is that the guy who wrote it certainly acknowledged that idea could easily have had an impact on the choice of Berg. I mean, nobody tried to say, you didn't deserve it because the science you did was trivial or whatever. Without being too modest, people recognized the science that I had done was first class. The recombinant DNA thing is a thing with a big impact. It's easy to latch on to that and say that's what you give the prize to. I don't know if I would have gotten the prize for the previous work alone.

So, if you add it up, there's a first-class science background, there's some kind of association with a very important scientific breakthrough, and then a leadership role in trying to manage the impact of that breakthrough. When it's taken together, people say, well, okay. There's another point as well. The Nobel prize is limited to three individuals.

Hughes: And, they have to be living.

Berg: They have to be living. There have been a number of instances where people were passed over for the prize because it would have exceeded three. In other words, there were four people that contributed equally, or this one person was unequivocally associated, and there are three others, and these three don't get it. In fact, such a thing actually happened. Phil Sharp and one other person, Richard Roberts, got the prize for discovering introns. Now, the person at Cold Spring Harbor who actually discovered the anomaly that led to the insight [Tom Broker] didn't get it. And when people talked about why he didn't get it, it was explained that it was because he and his wife had collaborated on it and that would have made four people.

Hughes: That's tough.

Berg: Okay, so now the question is, why didn't Cohen and Boyer get it instead of me?

Hughes: Or, why didn't Berg, Cohen, and Boyer get it?

Berg: Because, the Nobel Committee believed, I think, that the critical and important contributions to recombinant DNA were cloning and sequencing. Cloning without sequencing is trivial. Sequencing without having specific cloned pieces of DNA to sequence is also useless. Developing both were technical feats, and the two together are what really made the molecular genetics revolution. People cloned DNA and sequenced it. Okay? So, why not Cohen and Boyer, and Gilbert and Sanger? That's four. So, you could say, one compromise is not Cohen and Boyer, but Berg. It's difficult to know what the thinking of the Nobel Committee was.

Hughes: In speculating how the biohazards controversy may have elevated you in the Swedish consciousness, I wonder if Cohen's and Boyer's commercial ventures worked to their disfavor in the eyes of the Nobel committee?

Berg: I don't know. The secrecy that shrouds the decisions about the Nobel are remarkably well kept. The Swedes have managed to keep that process unpolluted by leaks. There may be speculations, and there are people who try to hype themselves or somebody else by saying they're being considered, etc. But, what I know from my Swedish friends who are involved in the process, it is very tightly guarded.

If somebody were to suggest to them that there were extraneous issues that were considered or played a role, they would deny it vehemently. People involved tell me they spend the entire summer researching a few candidates, going back through every paper, every collateral paper; trying to document priority is very, very important to them. In the end, this whole process winnows down to a few names, and then it's the respective Nobel academies that vote on them.

Recommendations from the individual committees have been reversed; that is, committees have come in with a recommendation, and the whole academy has rejected it and selected another candidate from the small group. So, who knows? Yes, in the final vote of the academy, it's quite conceivable that, without anybody conceding it, sentiment plays a role, because they all have to vote. And, if some guy says I admire this person for what he stood up for and what he did, it could sway their vote. But, I don't think those issues would have any impact at all with the screening and review committees.

Influences on the Choice of the Nobel Award

Hughes: I thought of Cary Mullis when we were discussing the fact that the prize is usually given for a body of research.

Berg: In that case, he had done nothing before.

Hughes: So there are exceptions to the rule.

Berg: Michael Smith, who was the other guy who got the prize that year, made one contribution when he was a postdoc of Khorana's. He hadn't ever distinguished himself in other ways; I've known him for a long time. The idea of being able to make targeted mutations in DNA the way he did it certainly has had an enormous impact. And, my guess is, that's why he got it. If you ask, was it a great intellectual breakthrough, a great intellectual inspiration, the answer is no.

Hughes: Why did the most preeminent prize in science go to two technological achievements?

Berg: I don't know which ones you're referring to.

Hughes: I'm referring to PCR and mutagenesis.

Berg: People have speculated, and I have no insight at all, as to whether there are contending forces on the Nobel Prize decision. Some would like to make it more practical; some favor more theoretical. Some favor more basic, others more applied. It almost seems to alternate. Like one year it's a group of physicists who developed a machine, another one it's a cosmologist who has come up with a grand theory.

And the same thing has happened in biology. When I say basic, it could be the crystallization and determination of the structure of a protein. And then the next year, it's somebody who has developed a whole series of compounds that are promising for cancer chemotherapy. Those two are very different kinds of intellectual activities. I don't know whether within the academy they debate this kind of issue.

Nobel's objective was to reward advances in science that had an impact on society, and he suggested that it be for a discovery in the year of the award. Well, very few of the prizes have been given for things that have had impact in that year, because, rightfully so, some period of time is needed to see whether the discovery is just a flash or whether it has some lasting effect. And so you have to wait.

Yet More on Recombinant DNA

[Interview 4: November 5, 1997] ##

Terminal Transferase

Berg: The existence of terminal transferase was long known. It was a curiosity, because it was the first enzyme that actually synthesized DNA without a template. That's not quite true. Kornberg had shown that DNA polymerase would synthesize some kind of funny polymer without a parent template. But, in this one terminal transferase, you needed a primer. You needed DNA, and all it did was add nucleotides on to the ends.

My recollection is that in Bollum's work it wasn't known if transferase would add on to the appropriate 3-prime end of a double-stranded DNA if it was blunt. It had to be a protruding primer end; that's what Peter Lobban essentially discovered and quickly related to us.

Creating Permuted Linear Molecules with "Tails"

Berg: In fact, I thought a little bit about it after our conversation. Our original plan was to open the SV40 circle at random sites, because we knew we were going to put tails on the ends. So, it didn't make any difference where we opened it. And by cleaving the DNA at random sites, we would have a population of linear molecules that were permuted, that is, they would have different ends because they were opened at different places. That would minimize the risk that where we were actually making the attachment to the other molecule would interfere with some essential function in the SV40.

So, we spent a lot of time using an enzyme called pancreatic DNase, DNase-1. It had been discovered by others, and we concurred that if you do it in the presence of manganese, you make primarily a single cut, and then it stops. So, we were cutting with pancreatic DNase, making linear molecules with different ends, polymerizing A's and T's on to the end, and then joining them together. But when John Morrow discovered that EcoR1 made a unique cut, we didn't know exactly which gene the cleavage was in. It turned out it was in the gene that specifies the capsid protein.

If I remember correctly, we used the R1-cut linears, because they were always uniform. And because they were uniform, we knew that they had 5-prime protruding ends, and that's not the kind of thing that you want this terminal transferase to be adding to. We needed a way to remove the 5-prime extensions to leave 3-prime extensions, and that was done with an enzyme which had been discovered at Stanford, called exonuclease-7. That enzyme specifically degrades double-stranded DNA starting at the 5-prime end and going in, therefore leaving 3-prime extensions. So that was essentially the strategy and how we evolved to it.

Choosing the Best "Tails"

Berg: The only other thing that I forgot to mention was, we spent a lot of time trying to figure out which were the best tails to use. We originally started out to ask whether G-C tails were the best to use. It didn't make any difference in principle which you used if you wanted so-called cohesive ends. But it turns out it was very, very difficult to polymerize G's onto the ends of DNA. There was a standing joke in the department that if you're going to have any trouble with anything in DNA, it's going to be with G. People tried to make dGTP and that always failed and the others worked well, and so on and so forth.

The reason why we were having trouble is G forms internal polymers with itself. And so if you polymerize onto the end and make a long tail of G, the chances are it is going to form some kind of funny structures. In fact, G-C forms triple-stranded structures. When that was discovered, we realized using G-C was causing bad things. So we went back to using A-T. I don't think we ever published the ins and outs and failures because we published in *PNAS*, and *PNAS* has a limited amount of space. We went right to the direct thing [A-T]. Actually, Bob Symons, who was an Australian visitor, did a lot of the work trying to produce these G-C tails.

The Jensen et al. Paper and *Biochemical and Biophysical Research Communications*

Hughes: Have you said all you care to say about the Jensen paper?

Berg: Other than I didn't know it existed. I never heard of it.

Hughes: What about the journal itself?

Berg: BBRC [*Biochemical and Biophysical Research Communications*]?

Hughes: Yes.

Berg: I was an editor of it for a while in its earliest days. I know I wasn't an editor at that point. Although, my memory--. It started out as a journal presumably to allow short hot new findings to be published. It certainly did that for some things. But after a while, it accumulated a lot of junk. I mean just things that people might have identified as critically hot papers the way they're referred to today. I went off it [as editor] because it was getting to be a nuisance. You see, the articles are quite short because of limited space. They were reproduced from your submitted text, thereby circumventing the need for redactory and all of that business. It was intended as quick publication; quick because they would just photocopy what you sent them. It never had a very good reputation. It was viewed as "bio quickies". I just decided I didn't want to be on it anymore. I don't remember exactly when it started.

[Berg indicates Jensen paper] This is volume 43, 1971, so you can see it must have started way back in the sixties. It came out every month, and it was just a nuisance. I don't think I ever read the bio quickies after I left the editorial board. I mean I rarely look at it. But people do read it, do refer to it, particularly people who are worried about somebody scooping them. There's a journal that publishes just the table of contents of various journals. So, people have resorted to essentially skimming the titles. I don't remember ever seeing the Jensen paper.

Hughes: Does the fact that Jensen et al. chose to publish in this journal suggest that they knew that they had something hot? You characterized the journal as a place for hot papers.

Berg: No. It had two features, one is you could submit short communications, and they were published quickly. And, they were almost unreviewed.

Hughes: The summary to their paper doesn't mention commercial applications. I wonder if they knew the significance of what they had done?

Berg: Actually, the last sentence is interesting. "Polynucleotide ligase"--DNA ligase--"does not covalently join the single strands of these synthetic catenanes." In other words, if you put A's and T's on the ends, and then they join, there are going to be gaps

because there's no way to control how long the tails are. So you can have twenty-five on this one and ten on this one, and they'll form ten base pairs, but then there's a big gap. He obviously didn't appreciate that since he couldn't control the length of the homopolymer tails. That's why we and Peter ended up realizing that when you hybridize the two and they join, DNA polymerase and the four triphosphates can be used to fill in the gaps and ligase to seal them.

Hughes: Jensen hadn't done that?

Berg: I haven't read the paper, but in the last sentence, it seems to indicate that he's unaware that there are gaps, which would be amazing. Oh, it says, "The catenanes formed by mixing T7 DNA which contained synthetic homologous ends were apparently not as well formed as those which occur in nature... We have been unable to catalyze covalent joining of our T7 DNA catenanes using *E. coli* polynucleotide ligase, even though numerous experimental conditions were used including variations in temperature, salt concentration and enzyme-substrate level." It never mentions the fact that the ends are unlikely to have been perfectly matched.

"Catenanes of T7 DNA molecules with various lengths of homopolymeric ends were also not joinable." Last paragraph: "Ligase joining during concurrent repair DNA synthesis was also attempted under conditions identical with those described by Goulian and Kornberg." But, these were unsuccessful.

This was submitted March 12, 1971. I don't have my notebooks to know where that stood in terms of our work.

Hughes: I don't remember when the Jackson, Symons, Berg paper was submitted.[1]

Berg: [Berg scans publications for submittal dates] These are all November '72.

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[1] July 31, 1972.

The Stanford Biochemistry Department's Industrial Affiliates Program

The Chemistry's Industrial Affiliates Program [IAP]

Hughes: What I really want to talk about today is the commercialization of the science. I thought that the place to start is with the biochemistry department's Industrial Affiliates Program. Does that seem appropriate to you?

Berg: Yes.

Hughes: In 1970, you wrote to Carl Djerassi saying that you were "taken", that was your word, by the brochure he had sent to you on the Industrial Affiliates Program in the Department of Chemistry, and that you were interested in starting something similar in the Department of Biochemistry.[1] Well, it apparently took almost ten years, because the first time any such idea is mentioned in the faculty minutes is in 1979.[2] The program didn't get off the ground until 1980. Was there a reason that it took so long to get going?

Berg: I had just become chairman of the department in 1969. So in 1970, if that's the date of that letter, I was charged with the responsibility of helping develop resources for the department. It struck me that the chemistry department had hit on a mechanism which was really very interesting because it generated a lot of money.

You realize that in 1970 our department did not have something it could go out and sell as easily as it did in 1979. We were a good biochemistry department. We were very highly thought of. Maybe many people would have thought we were number one. But the question is, what did we have to offer to industry?

By contrast, chemistry is cranking out graduates who go into the chemical industry, and more likely the chemistry department is doing research that's much more relevant to the chemical industry and the pharmaceutical industry. Carl Djerassi is a terrific

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[1] Berg to Djerassi, May 25, 1970 (Berg papers, Green Library, Stanford, S358, box 2, folder: 1970 A-H).

[2] Minutes, faculty meeting, Department of Biochemistry, October 17, 1979 (Arthur Kornberg papers, SC 359, Green Library, Stanford, box 5, folder 1979).)

salesman, and given his connection with the pharmaceutical industry, he was able to actually organize at one point forty companies at $25,000 per. So [Chemistry was] pulling in a lot of money. We didn't have anything like that, but we were well funded. Part of the resistance in the biochemistry department was, who needs an IAP!

Broaching the Idea of a Biochemistry IAP

Hughes: Do you remember that you broached it to the department after the interaction with Djerassi?

Berg: Oh, yes, I'm sure I did.

Hughes: If I remember that letter correctly, one of your arguments is that the federal funding of science is dropping off. Industry has benefitted all along from academic science; it's time for it to pick up some of the financial burden.

Berg: That's right. That's essentially what my interpretation was. [Berg skims copies of archival documents which Hughes has collected.] Okay, it gets picked up by Arthur Kornberg in 1979.[1] So, the only thing you have is my letter to Djerassi that's as early as 1970.

Hughes: That's right.

Berg: Also, there's a '67 letter in which Kornberg planned to visit at du Pont, and they wrote to him about that.[2] I remember Arthur coming back and just being totally disillusioned by the attitude of industry, and the way they did research and so on. Arthur didn't know anything about the affiliate program in the chemistry department.

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[1] Memo, Arthur Kornberg to Dale Kaiser, September 10, 1979 (Kornberg papers, SC359, Green Library, Stanford, box 5, folder 1979).

[2] Burt C. Pratt to Arthur Kornberg, October 11, 1967 (Kornberg papers, SC 359, Green Library, Stanford, box 26, folder: 1967 A-L).

Stanford Relationships with Industry

Berg: I learned at that time that Stanford had forty Industrial Affiliates Programs going in the university.

Hughes: In 1970?

Berg: I think so. Stanford was really a very entrepreneurial place. The aeronautical and engineering departments all had these ongoing relationships with companies, and that, I think, reflected Stanford's close relationship with industry, which led to Silicon Valley. The faculty were all involved, so departments nurtured these kinds of relationships, and they were allowed to blossom unfettered. If somebody was entrepreneurial enough to go out and create a program, nobody kept tabs on them as far as I know.

My recollection doesn't allow me to tell you that I brought it up to the department. The letter actually mentions something about a decrease in federal funding. I remember we had a crisis during my chairmanship when there was a threat that the training grants were going to be dropped.

Hughes: Right, I remember seeing a reference to that.

Berg: And the training grants were vital to our program. There was this threat that Congress was going to do away with training grants. I went to Washington and lobbied, and so on and so forth. And, probably it was in response to that that we were looking for new sources of funding.

Hughes: Which you didn't come up with?

Berg: I don't know the dates well enough.

Program Project Grant, Institute for Research on Aging

Berg: But, one of the things that the department landed was a program project grant from the Institute for Research on Aging. The department banded together four, five, six people, and we then made a proposal to the aging institute, and they funded us. It only terminated about two years ago. We held the grant for about four cycles of five years each. So we may have well have gotten it during this period. We have notes in the department; you could find out when we started the aging grant. The aging grant brought in a fair amount of money.

But what happened is, the politics changed, and Congress did not disband training grants, so we never lost the training grant. So it's conceivable that the reason nothing ever happened out of this first notion about the affiliates program was that the department was well funded. If the training grant was secure, my guess is we would not have developed sufficient enthusiasm for doing the affiliates program.

Sentiment Against IAPs

Hughes: But it wasn't opposed because of any feeling in the department that an affiliates program wasn't appropriate?

Berg: There were those feelings; there were some. I know that after we started our program, and I went around the country, and when I'd be visiting at Yale or at Cambridge, I mentioned the affiliates program, and some people said, That's not a thing that academics do.

I always justified it that we were doing was what we normally do here: we teach. We weren't making proprietary contracts with our affiliates. We weren't giving them first access to any discoveries. All we were really doing was providing expertise that was keeping them abreast of what was happening in their field. And, I saw that as an educational mission. We were just getting our tuition in different ways; we were getting it through companies.

Functions of the Biochemistry IAP

Hughes: I remember seeing in one of these documents that the point of the program was not to provide specific information for specific industrial programs, but to provide general knowledge, as you're saying, in this field in which you were expert.

Berg: That's right. It gave companies access to faculty. That is, the original thing was they could send somebody to visit the department each year to meet with faculty and students and postdocs. We would arrange for them to have sessions with students and postdocs for the purposes of their attempts to recruit, and we would send them preprints of our papers before they were published, and they would get these quarterly. And, at the end of the year, they would get a bound volume of all the

departmental papers. They had no proprietary rights to any discoveries, materials, or inventions, or anything like that, and that was it.

Once we mounted the program, everybody in the department was really strongly committed to it. Everybody agreed that they would go and visit. Furthermore, each company would have a visit from somebody in the department who would spend the day talking to scientists and give a seminar on their own research, and so on.

I dubbed this the Friends of Biochemistry, because that's the kind of affiliation I was hoping to sell and also would expect to have, namely, we're going to help you; you're going to help us. The notion of obligation wasn't ever really broached as a selling point because I think most of these companies eschew that kind of notion. They say, "We pay our taxes, and that's funding you guys." But, the idea was that it was mutually beneficial. They would get information. And, the reason we were able to sell it was, by 1979 Stanford biochemistry was one of the leading groups in genetic engineering.

Hughes: Right, and that's what they wanted.

Berg: And that's what they wanted. Many of these companies were totally in the dark about the developments. They were really grasping at straws, and that's why there was momentum for small companies. Guys who had ideas about what they could do with recombinant DNA essentially didn't go to big companies, because [the companies] didn't understand it.

Hughes: Were companies in the affiliates program less likely to start there own recombinant DNA programs because they had a relationship with you? Or was it an incentive?

Berg: It was more likely an incentive. The invitation to our affiliates to attend our retreats where they actually heard about the ongoing research didn't come until later. So, I can't cite that as something that would have spurred them on. Because if they had attended our retreats, they would have seen that all kinds of exciting things were happening which they were not part of.

Hughes: You're talking about the departmental retreats at Asilomar?

Berg: Yes, that came later.

Hughes: Yes, I have an agenda somewhere.

Launching Biochemistry's IAP in the Late 1970s

Berg: We began to discuss an affiliates program in '78 or '79. By that time, I think Bob Lehman was just coming to the end of his five years as chairman. We were doing five-year terms. I stepped down in '74. Bob Lehman would have done '74 to '79. So I suspect it was right at the very end of his tenure that we began as a department to become enthusiastic about doing this. And then Dale Kaiser became chairman. And so it was during his tenure that the program actually got underway.

Hughes: Why had members of the department become enthusiastic?

Berg: Well, probably two things. One is, given the notoriety of our department, we were now more confident that we had something to sell. Before, people were less confident, other than our general renown, that we had anything to offer to commercial companies.

But, when it became clear that we were quite out front in the area of genetic engineering, we felt we really had something to sell, and it would be negligent perhaps to not take advantage of that. We all made that same argument; we really could use the money. I think we may have brought up what Chemistry was getting out it. And having free, undesignated money; most grants designated how the money was to be spent. We didn't have a bank of free money. And so here was a way to accumulate money that would guarantee security of the department.

I think everybody bought into it. I don't remember that there was anybody who was opposed to it. And, as I say, we all committed ourselves to that list of offerings that we were prepared to do, and that meant [each faculty member] making an effort to go visit one of the companies. Most people saw that it was not a big deal because they were traveling a lot. So, on some trip East, they could stop and visit company x, y, and z.

Increasing Commercialization of Academic Biology

Berg's Prior Refusal of Corporate Consultantships

Hughes: You at Stanford were prime scientific movers of recombinant DNA. You must have been aware of people profiting from commercial application of recombinant DNA technology. Was there motivation to profit from it yourselves?

Berg: No, not at all. In fact--

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Berg: --I had stayed away from any kind of consultantships, even though many companies had come to me and asked me to be a consultant. And, I think they had probably done that to Arthur as well. And except for Arthur's connection with ALZA, which was largely through his friendship with [Alejandro] Zaffaroni and whatever other little companies Zaffaroni created, Arthur didn't have any consultantships, as far as I know, with any other companies other than those related to Zaffaroni.

I had a second reason for not accepting consultant positions. I had been a central figure in the "ethics debate," the safety debate, and, given the public policy debate that was going on in 1975, '76, '77, I thought I would lose my credibility in terms of the position that I took if I was also involved in some commercial enterprise.

It seems obvious that if you're working for a company, as I see now, you have certain obligations or interests, and those can easily influence and color your decisions about whether you think some things are right or wrong, appropriate or inappropriate, and so on. So I decided I did not want to do it. I was asked by Dave Baltimore to join one or another commercial groups, but I chose not to.

But, then I began to think a little differently about it, although I didn't do anything commercial until 1980. Dave Baltimore said, "Look, this field of genetic engineering is going to blossom and is going to move ahead. If you participate in it, you have a greater chance of influencing that it does it right and properly and ethically than if you stay out of it." I agreed that was a reasonable argument. But, nevertheless, I still said no to most things.

Berg Reconsiders Corporate Connections

Berg: It was probably about that time that the whole DNAX [Research Institute] concept got started, and we can go into detail about that. But that was the first time that I at least conceded to myself that this was an interesting proposition. Probably had it not been Zaffaroni [making the proposal to found DNAX], I would have said, "No way." And had it not been in collaboration with

[DNAX co-founders] Arthur and Charlie Yanofsky, I would have said no.

So it had a different flavor. Here were colleagues I really respected, had worked well with, with the guy who was going to do the business for us, who was a man I admired in many ways, and so I finally said okay. So DNAX was the first time I conceded that my stand-offish attitude was probably outlived. By that time, the [biohazard] debate had more or less disappeared; in 1980 things were blossoming.

The Recombinant DNA Controversy

Berg: In the summer of [1976] when the guidelines were issued, it was a really hot debate. I was traveling around the country, meeting with city councils and with university boards, trying to put a lid on preemptive strikes at each of these places to foreclose the possibility of doing recombinant DNA research, in the city of San Diego, for example, or the University of Michigan campus. I was involved in all these forums. So during that period, I felt it was inappropriate to be involved in corporate ventures and didn't get involved.

Herbert Boyer and Genentech

Berg: But I also defended the guys who did, particularly Herb Boyer. I thought there was an opportunity to use the technology to do something that would be medically significant, and if Herb wanted to spend his time to do that, fine. The only thing I criticized Herb Boyer on, and a number of other people at UCSF, was that for a brief interval after they founded Genentech, they were doing Genentech's work at UCSF labs. I thought that was totally inappropriate. And there was a whole lot of stuff about guys taking their materials from refrigerators.[1] There was talk of a

[1] In 1999, Genentech and UCSF settled a case out of court regarding use of biological material allegedly stemming from UCSF in Genentech recearch on human growth hormone.

kind of warfare that went on at UCSF amongst Bill Rutter, Boyer, and their postdocs.[1] That I thought was bad.

Hughes: Because it was so intimately mixed up with the academic?

Berg: That's right. Because they were doing work for their companies in academic centers. That was totally inappropriate. But once the Genentech labs got built, and they had a place to work off campus and so on, I didn't have any problem with that. There was always the possibility of a fuzziness in the boundary between what was done at UCSF and what was being done at Genentech and how much was passing from one place to the other. And, from the university's point of view, they were losing "intellectual property" by people essentially walking away with it.

Hughes: How did you feel about that part of it?

Berg: I spoke out against it. That's the only area on which I was critical about the people who were moving in that direction.

Hughes: Do you have any opinion why UCSF happened to be so active in technology transfer?

Berg: Well, Herb Boyer was clearly one of the leaders in the development of the technology in the field and Bill Rutter was one of the leading people in biochemistry.

Hughes: So, do I gather from that comment that it's more personality, more individuals, rather than the institutional context?

Berg: Oh, yes.

Hughes: From what you have just told me today, Stanford might be seen as a better locus for commercializing recombinant DNA because interaction had been going on between industry and academia for decades. Whereas, to my knowledge, there is not much of that at UCSF prior to the recombinant work. So, it boils down to individuals?

Berg: Oh, yes, there's no question. Herb Boyer being approached by [Robert] Swanson was the catalyst. I don't know what went through Herb's head in regard to why he wanted to do it. Either Swanson was extremely persuasive, which he could be, or Herb wanted to get involved with something to make money. And, I think Herb's

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[1] For coverage of this controversy, see: Stephen S. Hall, *Invisible Frontiers: The Race to Synthesize a Human Gene* (Redmond, WA: Tempus Books, 1987).

childhood and background, a small town in Pennsylvania, is often cited; becoming rich and famous was something that any young American kid would aspire to. I don't know the reasons. But he certainly has cited that.

This is just an aside, but if you look at what Herb has done with his money, he has done some really tremendous things--gifts to UCSF, to Yale, and so on. But, he also enjoys collecting classic cars and has a big art collection. So he has enjoyed the wealth, for sure, and used it well in many ways.

William J. Rutter

Berg: Bill Rutter was a very entrepreneurial guy right from the beginning. I knew Bill when he was the treasurer of the American Society of Biochemistry and I was the president of the society. Bill was always into finance. He loved it. He was the link between the society and the people who were investing its funds. He was keeping track of our budget statements and how much money it was costing us to run the journal. Bill was an entrepreneurial, financially minded guy. He understood the stock market. And, he was a good scientist. There is no question that he did an unbelievably magnificent job of resurrecting biochemistry at UCSF. I suspect that early on, he also saw opportunities to apply what he knew.

Cetus Corporation

Berg: Cetus was another company that pre-existed recombinant DNA. It was founded by Josh Lederberg and by Don Glaser. They also had a very entrepreneurial businessman who probably drove that.

Hughes: Ronald Cape.

Berg: Yes. And we watched that. And at that time,[1] there was no recombinant DNA. What they were trying to do was use Glaser's skill at instrumentation to create a machine that could do very rapid screening and isolate mutants that were more efficient in producing antibiotics and various kinds of other activities. But

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[1] Cetus was founded in 1971, before the discovery of recombinant DNA cloning technology.

they had nothing to do with recombinant DNA at that point. But when the recombinant DNA thing came on, they quickly latched onto it. They set up Stan Cohen here with Cetus Stanford, and created something called Cetus Immune with Hugh McDevitt, I believe.

Hughes: Yes, and wasn't there an agricultural branch of Cetus?

Berg: In Madison.

They set up Stan here somewhere in Palo Alto, I think. I don't recall where Cetus Immune was located. As near as I can tell, they all failed.

Hughes: You don't know why?

Berg: I don't know why.

Hughes: Was Cetus one of the first companies founded to commercialize biological knowledge?

Berg: Amgen got started after the introduction of recombinant DNA. So I think Cetus is the first. Josh was still here as chairman of genetics. I don't know of any other biology-based company. But there may be.

Hughes: Syntex?

Berg: Syntex was a more traditional pharmaceutical company that moved here from Mexico. Zaffaroni was not an academic. But Carl Djerassi had worked at Syntex, and when he was recruited to come to Stanford he maintained his connection with Syntex.

Hughes: Is Cetus really the first company founded by biological scientists, not necessarily by people applying recombinant DNA?

Berg: Except for Syntex, I don't know of any other.

Hughes: Was it significant for the biotechnology industry that a company in the Bay Area was making a go of applying biology in the commercial world?

Berg: I don't remember that the creation of Cetus and its operation ever elicited some kind of admiration or emulation. It was really making use of Glaser's skill in gadgetry, in invention of machines, because all they were doing was just mechanizing and automating large-scale ways of looking at microbial colonies and examining them in some clever way.

I remember Don Glaser came and gave a seminar and got a big yawn. He was a very bright guy, very dynamic. But nobody that I recall ever said, "Hey, that's the wave of the future."

Stanford's Policy on Consulting

Policy Reassessment, 1977

Hughes: Stanford had a faculty consulting policy which well predated the recombinant controversy.

Berg: Oh, yes.

Hughes: But in March of 1977, President Richard W. Lyman delegated to the Board of Trustees the task of establishing limits for consulting activities.[1] I can't tell you whether or not before that it was university policy that faculty spend not more than one day a week consulting. But certainly that came to be by 1977. Was the 1977 policy prompted by what was happening in the biological sciences, or was this a more general problem at Stanford, not connected with recombinant DNA?

Berg: I don't remember any problems, certainly not in the biological or biomedical area. If there was any concern, my guess is that Lyman would have been more concerned about the engineering school, which had traditionally done a lot of consulting. There were also people, for example, in the education field who were doing a lot of consulting in various areas. I don't remember that there was any specific event that instigated Lyman to do that.

Hughes: I'm glancing through the document and I don't see anything about why Lyman's concerned.

Berg: He says the policy contains some ambiguities. "It is in the best interest of both the faculty and the University to clarify these ambiguities..."[2]

Hughes: There must be something provoking him to reassesss the policy.

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[1] Lyman to members of the Academic Council, March 18, 1977 (Arthur Kornberg papers, SC 359, Green Library, Stanford, box 5, folder: 1977).

[2] Ibid.

Berg: Well, I think what happened, and maybe it happened around that time. There was a guy at UC Davis--

Hughes: Ray Valentine?

Berg: Valentine, who helped set up a plant biotech company.

Hughes: Calgene.

Berg: Yes. There was a bit of a furor over the fact that Calgene gave Valentine a research grant. He was one of the founders and the principle officer in the company, and they gave him a grant to carry on the research on the campus, that is, in a university lab. That elicited a certain amount of eyebrow raising--was that appropriate? My recollection is that there was a certain amount of clucking that went on about that.

I think the University of California established a policy which was that faculty could not accept grants from companies in which they had a financial interest. And it may be that was one of the things that stimulated Lyman. Maybe that was one of the ambiguities. I don't have the chronologies-- When did Cetus Immune, and when did Cetus Palo Alto get created? The question was, was this a way of funding Stan Cohen's research, or Hugh McDevitt's? I think there may have been a little bit of a concern about this boundary between your own lab and the lab that you are presumably directing for a company.

Hughes: Well, certainly, Donald Kennedy was very concerned, and even testified in the House in 1981. He says in his testimony that Stanford's faculty Committee on Research had voted overwhelmingly to reject the university's equity participation in faculty research ventures.[1]

Berg: I was one of the people that met with a group of venture capital people, university people, and trustees to discuss this issue of whether the university should in fact get into the business of venture capital. Should it be using its funds to help faculty found businesses? I argued vehemently against it. I wasn't the only one. The venture capital people urged the university not to do it, saying that the university didn't understand it; it would be a disaster for them.

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[1] Statement of Dr. Donald Kennedy, President, Stanford University, Stanford, California. Subcommitee on Investigation and Oversight, Committee on Science and Technology, House of Representatives, Commercialization of Academic Research, June 8 & 9, 1981, U.S. Government Printing Office, pp. 6-28.

But, more important, we were more worried about the impact on the academic setting. When the university is in business with one of its faculty, what kinds of relationships does that create? Does a guy get more money? Does a guy get favored promotions? There were all kinds of things.

But, going back to '77, again without knowing the chronology of when these companies were popping up around here. I don't know of anybody else in 1977 who was doing any consulting. What did get prominence was in fact that here we had on our campus or in our vicinity two of our professors engaged in a company. Cetus was already an existing company. If somebody felt there was going to be some financial gain, the way to do it was to start a spinoff, start something from scratch, so a person got in on the ground floor for any financial rise. So, I think that was one motivation for creating those two companies, or the three.

The third was to place new companies in the locale where those people were doing their research, because they could easily have said, "Look, I haven't got time to go over to the East Bay and solve this problem," and so on and so forth. "But, if it's right in my backyard, I would be left to run the operation." I suspect that is some of their motivation.

The Shooter Committee on Conflicts of Interest

Hughes: I saw a reference to the Shooter Committee.

Berg: I was on the Shooter Committee. The Shooter Committee was one set up by the medical school.

Hughes: It's called the Shooter Committee on Conflicts of Interests. So, you see there really is a lot of turmoil around this topic.

Berg: That's right. You have to recognize that the previous academic environment was very permissive. Nobody kept track of anything. Nobody knew what affiliations anybody had with companies. Nobody checked on how much time they spent doing these things. So, it was really an attempt to step back and say look, something is happening; our faculty is becoming involved [commercially], and have been involved, i.e. the engineering people and chemistry people.

Maybe the fact that the University of California put a lid on one area of involvement energized Stanford to look at the whole problem. And so Lyman reiterated what he felt was the policy. It

wasn't that stringent. It said that you could consult for 20 percent of your time.

Hughes: One of the tensions was that the clinical people had to turn over their consulting fees to the medical school. There was no control of the nonclinical faculty, as you're saying; they just put the consultant fee in their pocket; that was it.

Berg: That's right. Absolutely. Now, you're ringing a bell. Henry Kaplan, who was head of radiology and was one of the most powerful and influential figures in the medical school, was always griping about the fact that clinical faculty could not accept fees or anything of that sort. They were expected to be full-time, and as full-time faculty, they were expected to turn over all earnings to the medical school. And, I remember, as chairman of the department, almost every executive committee meeting was Kaplan pounding on that inequity. He kept identifying how inequitable it was that basic scientists were beginning to develop these kinds of consulting relationships and could keep their consultant fees.

I remember Kornberg made this argument, "We'll take your salaries, and we'll give up our consulting fees. But if you consider the disparity in our salaries, you can't be complaining about that we're engaged in this kind of consulting activity." So, that sort of kept a lid on it.

The Shooter Committee had to really look at the whole issue. I was a participant in that committee. What was a conflict of interest? A conflict of interest in my view arose if you have a financial stake in some decision you're making, and you haven't disclosed that you have a financial stake.

But the more critical thing from our point of view was what I called conflict of obligation. In other words, you're a faculty member here; you're expected to be here full time; you're being paid a salary. You're here, and we expect you to provide the kind of intellectual heat and creativity that makes this [university] a great place. If your head is somewhere else, there is a conflict of obligation. If you're thinking more about something you're doing on the outside than what you are doing here, then you're lost to us as a valuable person.

And so, we had to look at the whole issue of what was conflict of interest, and that was easy. You could easily say you have to declare any financial interest with anyone. If you're in a position to order a piece of equipment, and you buy it from a company in which you have a big financial stake, that's a conflict of interest. But conflict of obligation was much more subtle. For example, you're traveling around the country all the time

giving lectures, you're teaching courses at university X, or some summer program, and you're not around here and you don't fulfill your teaching responsibility here. That's academic; that's not in any way a consultantship; that's not in this 20 percent time.

You serve on many boards; you serve on many government committees, but you're not around campus. So the question is, how do we view that? How do we view a person who disappears from the scene to write a book? Obviously, he gets financial rewards from that book. What's different about that kind of activity than somebody who goes off and consults for a company, gets paid by a company?

Then, the second thing was, should we be snooping into how much money people are getting for their consulting? The Shooter Committee said that wasn't our business. But, we put in place a reporting requirement, that is, every faculty member was required to report what their outside activities involved and estimate how much time they spent at them. And, we believed that by forcing people to write down something, they would be lying if they did more. I don't think anybody wanted to lie or disobey, but when nobody asked, it was easy to just say, "Okay, it's a little bit more this month than last month." But very quickly, you'd find that somebody was spending a lot of time away. But we also set down very clearly this whole concept of conflict of obligation. I think that's now part of the lore that's used in terms of evaluating.

Hughes: Well, how did you settle that conflict of obligation?

Berg: We just pointed out that this was an area in which we expected individuals to fulfill their obligation to the university. They were being paid full time to spend their time here and contribute to the life and activities of the university.

I've had this conflict argument with other people; David Baltimore and I have argued this. He probably spends 30-40 percent at MIT and 60-70 percent of his time elsewhere doing great things. He said, "I am carrying the banner for MIT. By my being on various committees and so on and so forth, I am representing MIT and fulfilling an obligation to keep MIT at the forefront." So, people had different perspectives on this.

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Berg: Some believe that benefits accrue to the university from their participation and consulting, bringing the outside world into the ivory tower, knowing where the cutting edge is, what some of the problems are, making opportunities available to their students.

Things like that. I mean, this 20 percent time has been justified in many different ways, and it exists at Harvard, Yale, and MIT, and most other [universities]. It was understood that consulting was not to exceed a day a week, but nobody kept track, nobody asked. I think most universities now require faculty to fill out an accounting of whom they consult for and roughly how much time they spend at it each year.

Hughes: And who sees those reports?

Berg: It goes to the dean. But, if you ask me does the dean ever look at it; does somebody in the dean's office look at it? I don't know. They keep it on record. I think it's there largely should some abuse arise. Then someone can go back and say, "Look, you said that this year you were not going to spend more time [consulting]. Here, we now find out that you're involved in about five things. You're the owner of a company, or you're co-founder of a company, and you're spending a lot of time as a company officer." So, it was largely there just to have a record, which probably could be used when abused. But I don't think anybody actually reviews these reports.

IV DNAX RESEARCH INSTITUTE OF MOLECULAR AND CELLULAR BIOLOGY, INC.

Earlier Commercial Ventures

Hughes: In the second oral history that you did for MIT, which was with Charlie Weiner, you said--

Berg: What year was that?

Hughes: 1978. You did an earlier one in 1975.

Berg: Yes.

Hughes: --that you didn't at that time want to be involved with a company. I quote, "I have told at least four or five different companies that I will not participate as a consultant."[1] DNAX was founded in 1980, right?

Berg: Yes.

Hughes: How did you get involved?

Berg: Well, I've read Arthur's recounting of the story.[2] Both Charlie Yanofsky, and I have a slightly different story. [laughter] And it could be because we weren't aware of some of things that Arthur was doing or saying. First of all, Arthur was a consultant; he was actually a member of the board for ALZA. He was also a member of the board for some little spin-off that ALZA created to try to immobilize food dyes on various things, and that failed.

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[1] Paul Berg (second interview), April 17, 1978, p. 64. MIT Institute Archives, Recombinant DNA Oral History Collection.

[2] Arthur Kornberg. The Golden Helix: Inside Biotech Ventures. Sausalito, California: University Science Books, 1995.

Channing Robertson's Company

Berg: There was a guy named Channing Robertson who was the head of the chemical engineering department. He had the idea that the biotech industry was going to eventually want to produce products. Everybody was cloning things, and they were trying to figure out how to get them expressed. But, in the end, what had to be produced, if it was going to be usable, had to be produced in large quantities. And, being a chemical engineer, he was very tuned into how you go about manufacturing chemicals.

What Robertson realized was that this was a whole new ballgame because proteins were going to be the products of these genes, and the chemical industry had not had any experience of how to produce large quantities of proteins. So he had been developing the kind of technology which allowed one to be able to grow bacteria to very, very high densities, much higher than any way you can grow them in a flask.

He could see that technology was going to be advantageous since bacteria at that time semed to be the organisms that were going to be producing these rare proteins. If you could grow them to higher and higher quantities-- He, not being a biologist, needed some help, and he came to see Kornberg. He also talked to Charlie Yanofsky who was very experienced in growing bacteria.

Robertson told us about this notion he had of forming a company, and that Stanford was in some way interested in becoming a partner. Not bankrolling the whole thing, but in fact retaining some kind of interest should this company do well. He went out to a lot of chemical companies and raised a whole lot of money. I don't think Stanford put any money into it, but Stanford retained some form of interest which I just can't remember. He had a venture capital firm here on Sand Hill Road, Palo Alto that would be a principal investor. Robertson came to us and asked if we would co-found this company with him.

Hughes: We being?

Berg: Kornberg, me, and Yanofsky. We were to be the scientific backing for a venture into the next stage of genetic engineering. Instead of doing it in test tubes, we were going to do it in factories. And we said okay. This was about the time when I began to reconsider my earlier objections to being involved in commercial biotechnology. Zaffaroni was not even involved in this venture.

Hughes: Was this in 1980?

Berg: Probably the year before. Could have been '80, but I think '79 more likely. We thought it was kind of an interesting opportunity and that Robertson had a fascinating idea for the kind of technology he wanted to develop. He was the engineering expert. And so we said okay. Then, we went to meet with the venture capitalist to discuss what our stake would be in this company.

We were so sickened by our discussion with this guy that we came out of there just saying, "Pretty slimy character." Although he isn't a slimy character, I've learned since. But what they were willing to give us in terms of stock options and blah, blah, blah, seemed marginal. But, since we had never been involved in this kind of business that wasn't so clear.

Alejandro Zaffaroni

Berg: So Arthur went to ask Alex Zaffaroni whether that was a fair deal. That's my recollection. Arthur says he came to it in a very different way. But I remember that Charlie and I thought of asking Zaffaroni whether this was a conventional standard, fair kind of grant. And Zaffaroni just laughed and thought it was ridiculous and said, "If you guys are really serious about wanting to get involved with a biotech company, I'll form one and you can join me."

Alex then told Arthur and us that he had been approached and had actually considered becoming an investor and a guiding light for a biotech company that Harvard might start. He had been approached and asked whether he would be interested. He had consulted with Arthur about it--which may be, but certainly we were unaware of that conversation--but he said he turned them down. When he realized that we were interested he began to consider doing something with us.

I didn't really know Zaffaroni well, but I knew him through Arthur, and I knew Arthur had the highest regard for him. He was clearly a very innovative and creative entrepreneurial guy. He said he would do all the business, and what he wanted us to do was to provide the scientific energy behind forming this company.

Zaffaroni may have told us what our financial stake would be if we started this company. It put the other one to shame. The same attraction that had led the three of us to even consider joining with Channing Robertson was there in thinking about DNAX, except that now, with Zaffaroni, we had somebody whom we respected and had confidence in. The Robertson company seemed more

ephemeral, although there was going to be a lot of other companies involved along with the university. I don't remember that I particularly thought that was bad because their stake was not anything like what I had argued against before. It was a different nature. Again, I don't remember the details.

Hughes: So you got out of the arrangement with Robertson?

Berg: We got out of it.

Hughes: Did anything happen?

Berg: We went to Channing and told him that we had this alternative opportunity, and we were much more attracted to that. For one, it got us into an area that was more closely related to our interests, which was how to use recombinant DNA to make a significant medical product, rather than commercial quantities of proteins. Robertson's company was formed and went along for a little while, and then it failed. I think the companies that were interested from the beginning began to recognize that there were other kinds of technology available to achieve the same ends, whatever. But it eventually failed.

DNAX

Initial Research Focus, Recruitment, Science Advisors

Hughes: Had DNAX been refined any further than that? What were you hoping to do with recombinant DNA technology?

Berg: Well, we had several brainstorming sessions. And we decided that immunology would be the focus. Once we were engaged, Alex Zaffaroni said, "I want to bring in a bunch of other people," one of whom was a guy named Ed Haber, who just recently died. He was a professor at Harvard. And then he engaged a bunch of people who had been involved in ALZA, about five or six other people.

The first question was, who were going to be the scientists? We were implored to try to convince some of our postdocs to join DNAX. The first group we approached were postdocs, the first being Kenichi Arai, from Arthur's lab. He had probably been Arthur's best postdoc; he was a dynamo. But he had gone back to a position in Japan but his wife, who was also a scientist, couldn't have a position there. Arthur contacted him and said, "How would you like to come back?" He did. There was a postdoc of Charlie

Yanofsky's, Gerard Zurowski, who had gone to Australia with a Queen's Fellowship. He came back. Lee Hood, I think, was an advisor. So he sent us one of his people, Kevin Moore, who is still at DNAX.

Hughes: It was an impressive group of advisors.

Berg: This is typical of Alex Zaffaroni; you engage a lot of big names, to impress potential investors and for recruiters. But a lot of the advisors contributed very little.

Ed Haber and the Engineering of Monoclonal Antibodies

Berg: The interesting thing was that Ed Haber was a cardiologist at Harvard. One of the drugs that he pointed out was very useful, was Digoxin. Older people, he said, frequently overdose on digoxin. They forget that they just took their pill and they take another. Or, some kids eat grandpa's pills and they go into coma. What he had done in order to treat people like this was to develop a monoclonal antibody, which he was using to inject into such people with remarkable results; like within twenty minutes to a half an hour, they would be out of the comatose state, get off the bed, and go home.

Hughes: Where would he have found the monoclonal antibody?

Berg: He made it. He was an immunologist by training, a very good one. But he was a cardiologist by profession. So he made a monoclonal antibody against Digoxin by attaching Digoxin to an inert carrier. You inject the carrier and then you screen for antibodies that are directed against the chemical entity to which you attach it. You remove all the antibodies that are to the inert carrier, and then you get antibodies very specific against Digoxin. And then, you try to get one that has a very high affinity for it.

What Haber found was that he could clear the body of Digoxin by just injecting the monoclonal antibody. Antibodies come with two kinds of chains, heavy and light chains. He claimed that you could take the Digoxin antibody apart and it would reassemble. I think he also had shown that you could make what they call an Fab fragment, which is a fragment of the antibody but it contains the combining sites. Fab fragments have all the same kind of specificity and affinity for these ligands, antigens. But they're much smaller and they are secreted or excreted very rapidly.

Haber had shown that the Fab fragments were even more efficient in being able to sop up the digoxin. They gained entry into places that the big proteins can't go. They're smaller bits; they go in and attach and eventually are excreted. So, we thought we could synthesize the heavy and light chains in bacteria by isolating the genes that encode the heavy and light chain proteins. And, since he had these cells that were making this monoclonal antibody, we could isolate the cDNAs from them and engineer them so that we could make heavy and light chains in E. coli. And, the heavy and light chains could be prepared in large quantities, then we could reconstitute the active antibody.

We knew it wasn't possible to engineer the structure of the antibody combining site directly, but we could change the structure of the gene at will. And we could make antibodies with very different kinds of specific affinities. So, the concept had emerged--Zaffaroni gave it a name, but I can't remember the name of it. But you could imagine that you could start making antibodies that would be used in the chemical industry for extracting things. You could make antibodies specific to anything. You could use them like a first-aid kit. You can imagine, for every kind of toxic substance that somebody could ingest there could be a monoclonal antibody that would be injected into a person to neutralize the toxic agent.

Hughes: How far along was the technology? How grounded in actual science were all these ideas?

Berg: We knew you could make cDNAs. We had these brainstorming sessions. We sat around the table and said, "Okay, our first target ought to be to use genetic engineering to make specific antibodies, or parts of antibodies, engineered in ways to give them special properties, which could be used in a variety of commercial and industrial and medical uses."

Utilizing the Okayama-Berg Procedure

Berg: Now, my lab had just worked out a technique for being able to make full-length cDNAs. The earlier work that had been done by Tom Maniatis said made it possible to make cDNAs but they were rarely full-length, full-length meaning that they contained the entire protein coding sequence.

Hughes: Would that be Okayama?

Berg: Yes. The Okayama-Berg procedure was designed to guarantee that you got full-length cDNAs, even if they were very big genes. And so I said, "We have a technique for being able to use Haber's cells, extract the RNA from them and make full-length cDNAs that would encode the heavy and light chains." Charlie Yanofsky was the world's expert on regulating expression of genes in E. coli. We could then engineer these coding sequences into E. coli and make large quantities of heavy and light chains. Then, we would extract these heavy and light chains, purify them, then put them together, and then reform the antibody.

Hughes: Was it somewhat fortuitous that you three had major facets of the problem to contribute? Is that why you were involved initially in DNAX?

Berg: When we agreed with Zaffaroni, there was no clear plan of what we were going to do. Therefore, our individual expertise, other than being in a general area of molecular biology and molecular genetics, was not evident. It was Ed Haber who brought this as a possibility and said there was a market for these kinds of things. Then we began to say, well, how would we make antibodies? Well, the only way to make antibodies that I knew was to actually clone the genes. But not the genes because the genes are much more complicated to work with. Clone the cDNAs, express them in E. coli; Charlie knew how to do that. Arthur was sort of not involved because he wasn't an expert in any of these areas. But we did adopt the Okayama-Berg method as the principle approach to do this.

Okayama being Japanese sometimes writes English a little more like Japanese. And so, the method that was published was actually quite difficult for most people to follow because it was quite intricate. Again, like the first creation of the first recombinant, it required a lot of experience with enzymes and dealing with them in different ways. Most of the enzymes we had in-house.

Hughes: So, you had a corner on the market?

Berg: That's right.

Problems in Engineering Monoclonals

Berg: Kenichi Arai came from Japan. He immediately consulted with Okayama, and before you knew it, these guys at DNAX were cranking out clones with no problem. It turned out, they made heavy and

light chains, but they would not reassociate. Ed Haber had shown that he could reassociate heavy and light chains for other antibodies, but not this one. So here we'd gone through this entire exercise and everything worked perfectly; full-length cDNAs were being expressed in E. coli. But, they would not reassociate. We tried to make it so that both genes were being expressed in the same cell, and maybe they would reassociate under those conditions. We never could make a functional antibody.

Money was running out. Alex had raised some $4.5 million to get DNAX started. We didn't have more than about ten or twelve scientists working and we had used up all of this money.

Fundraising

Hughes: I know Arthur was involved in some fundraising trips.

Berg: Oh, yes.

Hughes: Were you as well?

Berg: I went on one fundraising trip with Alex to New York, where we met with the people from the Rothchild venture capital company. It was based in New Jersey. Alex and I met with their group of scientific advisors; tried to get them to invest. Sydney Brenner was their consultant. He put us at the top of the heap in terms of scientific expertise and prominence. He thought DNAX was a great, great idea, and so on and so forth. But, they never bit. In fact, it was one of the greatest disappointments I ever had. We never even got a response from them. We met with them, we took them to dinner, and we had this fancy talk. I thought we convinced them and that they were going to buy it. [laughter] But, we never even got a reply.

Hughes: Do you think they understood the science?

Berg: Oh, yes, they understood the science. Arthur, I think, met with somebody from Rothchild's on one of his trips to England. We never could find out why it didn't go. But, in any case, Arthur and Alex made several trips to Japan where Arthur had all kinds of contacts. A former postdoc of his, Omura, was a major figure for Takeda Chemical. So DNAX was on the verge of going under.

Schering-Plough and DNAX

Schering-Plough's Research History

Berg: I think Alex went to Schering-Plough because the new management of Schering-Plough were people that he had known from Ciba-Geigy. Ciba-Geigy had been, essentially, the owner of ALZA; they bought ALZA from him. And so he went to see them to tell them about DNAX. And there was the almost extraordinarily fortuitous circumstance that they were looking for new directions. The company had been at its nadir in terms of successful products. They'd had a very successful antibiotic, which was going off patent. Their prospects were zilch; they had nothing in the pipeline. And, they had, during the year 1978-'79, conducted a strategic survey of what were the promising directions to go in in the future. And, they chose immunology.

To implement this new strategy in immunology, they created a laboratory in Lyons, France, largely because they were obliged to by the French government in order to protect their pricing capabilities. So they built a lab there and said this is where we're going to do immunology. And when they went out to try and recruit people to do it, they were really unsuccessful. Everything that they tried to bring immunologists into their research organization had failed, in part because their reputation was pretty lousy for research.

So when Zaffaroni presented them with the existence of a company committed to immunology, and they saw the board, and they saw the scientific advisors, and they saw that there was a team of scientists on board, they began to see that if they just acquired DNAX, they would have an instant presence in the field of immunology research. And so, after a few discussions, I went East with Alex to make a presentation to the scientific board of Schering. We thought they were just going to make an investment, keep DNAX separate. But then Alex came back and said their proposal was to acquire the whole thing. And, I can tell you there was a lot of discussion and resistance.

People thought that we had committed to building something, creating something special. We had this sense of ownership, and suddenly we were selling out. But, Alex was astute and recognized that in fact all the value that DNAX had would grow. Because, if Schering Plough and its fortunes were down, and they ended up, we would all benefit from that. So, we bought into it.

Scientists' Initial Reluctance

Hughes: What about the effect of the poor image research in the pharmaceutical industry had in the academic world?

Berg: Oh, there's no question that existed. That was part of the thing. We didn't respect their science. Frankly, not many of us even knew anything about their science. We didn't know that they had not been a very successful pharmaceutical company in generating research, in generating products. They were just some big enemy out there, part of the pharmaceutical industry which none of us had a lot respect for.

But, I think the principle thing is, or at least my recollection is, we felt like we were selling out, that we had started this [company] with a grand idea that we were going to create the next Syntex, or something like that. We had established certain criteria that DNAX had to meet: we had to have a very academic environment, that people were free to publish, that people would be able to talk about their work. All the kinds of things that were anti-traditional to pharmaceutical company style, we were rejecting. And, we said DNAX is going to be an open environment--

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Berg: --so that the scientist would not become invisible to the academic community and they would be able to move freely back into academia. If you were successful, you would be known. You would be publishing and so on. So, we were very concerned that through this acquisition, all of what we promised these people and the kind of style we were hoping to achieve would be lost, and that Schering would not honor that goal. But Alex, I think, was hugely successful in persuading them to leave DNAX as an independent research institute, to live by the parameters we had created for its lifestyle, culture, and everything that we had told people was going to continue.

Hughes: If he had not been successful, do you think you would have stuck with it?

Berg: Well, I think one would have had to see just how offensive it could have become.

J. Allan Waitz, DNAX President and CEO

Berg: In fact, we came very close to rejecting the arrangement, because once the deal had been consummated, we were sent a person from Schering-Plough to become the president. We thought, this was the beginning of the end; here was a guy who was going to instill his culture, their culture on us. But it turned out, he was totally swept away by the way we were, and Al Waitz became the great champion to the point of offending his Schering-Plough colleagues and masters by how he defended our way of life, and the way we were doing it, and what we were doing, and so on.

Hughes: I remember from Arthur's book the comment someone at Schering-Plough made to Dr. Waitz, "Are you working for them or for us?"[1] [laughter]

Berg: Al was terrific. He really got caught up in California, and our style, and the way we worked. He had a lot of influence at Schering-Plough, because he had been a leader in their interferon project. He had worked with the CEO, [Robert P.] Luciano, and so Luciano respected his views. Al protected us against a lot of other people.

Tensions between DNAX and Schering-Plough

Berg: As you might imagine, there was a lot of jealousy because we were allowed to work in one way on the things we wanted, and people there were required to work on company projects. And then, the quality of the science in the two places was a mismatch. I mean, there's no question, Schering-Plough was the pits, and people we had at DNAX were leading people. When DNAX reported what they were doing or tried to get collaborations going, they lost respect for the people at Schering-Plough. DNAX scientists didn't think Schering-Plough scientists were quite up to being able to follow them. So I think in the beginning there was a lot of tension.

Hughes: Another source of tension, I should think, would have been Schering-Plough's rather ambivalent, or maybe even negative, experience with Biogen. Remember that?

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[1] Arthur Kornberg. The Golden Helix: Inside Biotech Ventures. Sausalito, CA: University Science Books, 1995.

Berg: Yes. Their financial deal was, they made an investment in Biogen, and in return they got the complete license for the use of alpha interferon. That was what they bought; they bought a product. In order to buy a product, they had to make their investment. I think they had a 10 percent ownership. Al Waitz was on their board. Hugh d'Andrade was on their board. I don't think Schering-Plough was disillusioned with Biogen until quite a bit later. And in the end, they got a big financial return. Not only do they have a product which today is a three-to-four-hundred-million-dollar-a-year product, but in addition, their investment in the ownership of stock went up. So they did quite well.

But the DNAX thing was really more of a strategic investment, that is, if they had decided that immunology was going to be the core of their research program, and the entree into infectious disease, cancer, and things of that sort, they needed a strong core.

Hughes: Who were the main competitors in that area? Presumably other pharmaceutical companies had also targeted immunology?

Berg: Oh, yes. I think immunology was seen as a central player. I don't know who else staked their strategic future to the extent that Schering did. Big companies probably didn't have to. But, Schering-Plough was really groping. They didn't have anything, and the market knew that.

Hughes: Well, as you know, once the Schering-Plough acquisition occurred, there was a shift in the research agenda.

Berg: Yes.

Hughes: Would you like to talk about those decisions?

Berg: It's twelve o'clock.

Hughes: Oh, that's what that means?

Berg: Yes, my little beeper beeps.

Hughes: It tells you when you're hungry? [laughter]

Berg: No, it's there because this watch has to have an alarm setting. And so I conveniently put it at noon because I'm up. If I put it at seven o'clock in the morning, I don't want to wake up. So, I had to find some time [to program it to], and it always evokes some comment, "Oh, is that my time up," or something like that. But actually, I booked us for lunch, like I did last time. [interruption]

Maxine Singer was invited to the meeting that we had at MIT where we drafted the "moratorium", or "Berg", letter. But she was taking her kids to Disney World in Florida and decided that was more important than being at that meeting. But when we came out with that letter and were planning to have the Asilomar meeting, I certainly her to be a participant on the organizing committee.

Her husband, Dan, was at the Asilomar meeting and was very influential. As the history goes, the lawyers opened our eyes to our collective responsibility, obligation, and vulnerability to being sued, and so on and so forth. Dan was the one who organized the discussion that evening by the lawyers and helped to identify the people to invite, all of whom were very influential in the outcome.

ALZA and DNAX

Hughes: I have a news release of March, 1981,[1] which was before Schering-Plough acquired DNAX the next year. Zaffaroni announced that DNAX's strategy was "unique in combining three technologies--genetic engineering, immunobiology, and drug delivery systems." Now, the drug delivery systems was a Zaffaroni connection, was it not?

Berg: Well, one of the investors in DNAX was ALZA; $600,000 worth, I think, out of the $4.5 million that bankrolled the company. Zaffaroni's vision was that we could make proteins through genetic engineering, and that ALZA was going to develop delivery devices for delivering proteins. Remember, delivering proteins across membranes is not a trivial job. And, all of the delivery devices that they had made until then were delivering small molecules.

He foresaw that if you made therapeutic proteins, delivering them at known sites, known rates of release would be important. And so ALZA was supposed to be part of this kind of consortium that would design delivery devices for proteins. They still haven't done it. Delivering proteins is still a big, big challenge.

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[1] [News release], March 9, 1981. (Papers in the possession of Arthur Kornberg concerning DNAX and his book The Golden Helix, "Helix" carton)

DNAX's Original Product Goal

Berg: The whole thing that we were focused on was how to use the recombinant DNA technique to actually make designed immunoglobulins with very special properties. We talked about being able to make enormous columns, if you will, of protein molecules, mobilized on supports through which you could pour crude mining extracts. And they would extract the gold, or they would extract some very, very low abundance material because antibodies have an incredibly high affinity. And you could use genetic engineering to modify the combining sites of natural proteins to increase their binding specifity and so on. Alex actually got a patent. It was for high-affinity binding sites, or something like that. I forget what it was called.

[perusing documents] Dynapol was the other company [of Zaffaroni's] that Arthur was involved with. It was a spin-off from ALZA.

Hughes: Is that the company you were referring to?

Berg: Yes. I think Arthur says he lost his shirt on that. [laughter]

One of the important things that emerged after the acquisition, which really helped delay some of our fears about what was going to happen, was that DNAX was to be governed by a policy board. The policy board was to have me, Charlie, Arthur, Zaffaroni, and three representatives from Schering-Plough, and that balanced how things were to be decided. These policy board meetings would be held here in Palo Alto. Like a board of directors of a company, it would act as a board of directors for this common joint venture, and that, again, helped make us feel that Schering-Plough was willing to make concessions. Then, there were reassuring statements about East is East and West is West, and we're not going to try to blend the two. The personnel policies were different for DNAX than they were for Schering-Plough employees. So they clearly recognized that there was something special about DNAX and it had to be nurtured.

I shouldn't say they realized. They were taught, totally encouraged to believe that. [laughter] At every meeting, Zaffaroni, Arthur, and I would beat on them about the very special quality of DNAX and the risk of losing it. In fact, I remember when Alex was trying to talk us into going for this merger, he said, "What have you guys got to risk? Let's assume that they come in here and they transform DNAX into Schering-Plough West. You guys leave, what have they bought? They bought you. That's what the value is. There's no product; there's no anything; they

bought you. So in order to make their investment worthwhile, they have to enlist your compliance." And we realized that was true.

The Shift to T-cells

Hughes: There was a shift in research direction after the Schering-Plough acquisition. It was not going to be antibodies, but interleukins.

Berg: Well, what happened is in this strategic review they had, the part of immunology which Schering-Plough got sold on was T-cells.

Hughes: Who was pushing T-cells?

Berg: This was a guy named Harvey Cantor, who was a professor at Harvard, who was a good immunologist. He had been on this strategic advisory board, and he had persuaded them and the rest of the people that T-cells were the crux of the immune system, as it was understood in 1980. They are the cells that sense antigen first, and they elicit the rest of the immune response by secreting cytokines, interleukins as you call them. He had already done some studies to show that a certain cell line, when stimulated, produced all kinds of cytokines.

So when Schering discussed the acquisition of DNAX, they said they were not interested in antibody production; they were not interested in making these mickey mouse little things that could be injected to cure overdosing on Digoxin. They would be interested if we would work on T-cells. Nobody felt totally wedded to the antibody project. That was always viewed as being just to get us in the door, just to get the company started.

Hughes: Not much research on antibodies was already done?

Berg: It had been going along for a bit. It clearly wasn't succeeding, and we probably would have been confronted with making some new choices. But when they said T-cells in trying to identify these cytokines, it was clear we could translate the same technology, that is, cloning cDNAs. Within an instant, Kenichi Arai and those guys took the cell lines from Harvey Cantor and started cloning out cDNAs for all kinds of things.

The Expression Vector Technique

Berg: One of the major things they had adopted was a technique Okayama and I developed called an expression vector. Expression vector means that when you clone the cDNA into the vector, it's already in place to be expressed to make the protein. Whereas with normal cloning, you just clone it into a plasmid. After you've cloned it you try to recognize it by its sequence. But if you don't know anything about the gene sequence, and you're looking for a gene that makes a kind of protein that has a biological activity, you want a system where you make the proteins in the cloning operation. We had developed this expression cloning system, and that was probably the single most important contribution to the success of DNAX. DNAX cloned all kinds of cytokines.

Hughes: DNAX had this expression system when other companies did not?

Berg: Other companies did not. But very quickly others recognized that that was the way to go. So they developed variants, all of which had the same capability so that when you cloned the sequence, it was in a position behind the promoter so it could be expressed in animal cells or in any system you wanted.

The company Genetics Institute developed an expression system; actually it was a postdoc, Steve XX [Berg can't recall the full name] from my lab who had been there when Okayama developed the expression cloning system, who then went to Genetics Institute and developed a comparable promoter using adenovirus. We were using plasmid systems. Essentially, it took advantage of the fact that you could clone full-length cDNAs, and when they're cloned, they're put into a vector that expresses them. So DNAX was a new gene a month. I mean, it was really booming because it had developed a very good way of cloning rare things that had never been known before.

Recruitment of Scientists

Hughes: Whom did DNAX recruit?

Berg: There was the core group: Kenichi Arai because he came back from Japan, Gerard Zurowski came back from Australia, Kevin Moore from Lee Hood's lab, a few others. DNAX tried to recruit Okayama, because he was a whiz. But he came from a Japanese background where he thought he would be unlikely to ever get a job in academia if he went into industry.

Hughes: It probably would have been true at that time, would it not?

Berg: At that time, it might have been true. Today, it's not at all true. Kenichi Arai went back as the professor of biochemistry in the Institute for Science and Technology at Tokyo University, and Okayama went from being the professor in Osaka to Tokyo University.

Hughes: It sounds, from the way you've just described it, that the way these people came to DNAX was largely through personal connections.

Berg: That's right. And a lot of selling--selling in the terms that this was going to be a congenial, exciting, productive environment that would be every bit as academic as any academic position would be. They would be paid well and have a stake in the future of the company, and they were free to publish and talk about their stuff.

Research Freedom

Hughes: Also, and this really surprised me, DNAX scientists were given the opportunity to pursue their own research.

Berg: That's right. Within certain bounds. Somebody wanting to work on photosynthesis would not come to DNAX.

Hughes: There was somebody at DNAX working on photosynthesis.

Berg: There was, and Gerard had been doing it before.

Everybody was assured that they had a certain fraction of time, 20, 30, 40 percent of the time, that they could do whatever they wanted. And their other research was to relate to the principle theme of DNAX. But the principle theme was very broad. It was molecular immunology and subsequently became molecular immunology and cell growth control. Within those boundaries, people could do almost anything.

Gerard Zurowski, who was working in Australia, was doing some plant work. He came here and carried on the plant work for a while. But he got so caught up in the immunology part of it and the plant work eventually went by the wayside, and that happened for most people. The work was so exciting and successful that most people got drawn into it and left behind whatever thing they had in mind before.

Hughes: I spoke of the cloning gold rush. I saw some memos that stated that after a few years, there was a lull at DNAX and other companies rushed in and actually took the plums.[1] What was your feeling at that point?

Berg: Well, my recollection is, the first plateau was all these cytokines were being mined successfully by Immunex, which is another company, and Genetics Institute. There were a number of times that we won, a number of times they won. That is, they got to the goal first even though we were all competing for the same thing.

Cloning Cytokine Receptors

Berg: But then we came to the realization that the next big direction was to understand the receptors to which these cytokines bind. The way cytokines act is they bind to receptors that are on the cell, and then there is a signal transduction; something gets passed on to the nucleus, which then triggers the cell to do something to respond to that signal. What we began to realize is that just knowing the first part of it, what are the things that you put out as hormones, wasn't enough; we had to understand how the cell responded to those hormones, those signals. That's the crux of the immune response.

So, we had to pursue new goals. One of those new goals was to start cloning the receptors specifically for these cytokines, and ultimately to understand the pathway by which a signal transmitted from the receptor to the nucleus. And that, today, constitutes the principle mission of DNAX. What DNAX had as an edge was a very special skill in isolating and focusing on the rare cells of the immune system. In other words, you could take T-cells, but then there are subclasses and subclasses, and there are different kinds of B-cells at various stages. And all of them have unique patterns of gene expression. So if you can begin to enrich for certain cell types, you have access to certain kinds of proteins that somebody looking at the bulk won't ever find. And so that has been the DNAX's expertise.

Today DNAX is moving along working with cell types which probably very few people in the world know how to isolate, grow, and adapt. DNAX is looking for gene discovery in those cells

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[1] For example, see: J.A. Waitz to Policy Board Members, December 29, 1986. (Arthur Kornberg personal papers, DNAX 1987-1988 [sic])

under the assumption that if you identify the important protein in that system you might have an edge into a particular function, maybe a disease, and so on.

A Long Discovery Phase

Hughes: Arthur in his book writes about the congeniality between the people at DNAX and Schering-Plough. But I nonetheless suspect that there were some tensions. Did people like Luciano, the top guys, appreciate how long it would take from the discovery phase to an actual product?

Berg: They did. They told us right in the beginning that they did not expect to see any products from DNAX research for probably the order of ten to twelve years.

Hughes: How could they have known that?

Berg: If you take a strategic view to the pharmaceutical industry, you have to think long-term. It takes a long time from even the most promising discovery in the lab until you get something you can sell. And many things fall by the wayside. So they knew the game.

Hughes: From past experience.

Berg: Yes. I think what they were trying to do was to take the pressure off us. They wanted us to do cutting-edge research. They wanted us to be at the very frontier of this field, with the confidence that it was going to lead to commercial value. And they didn't want us to feel that we had to prove tomorrow that we had made something.

DNAX Benefits Schering-Plough

Berg: But in point of fact, within a very short time, Schering-Plough had patents on a lot of very valuable stuff.

Hughes: But an actual product wasn't on the market until about 1990.

Berg: That's right. But Schering knew that they had things in the pipeline that were potentially very valuable. But more importantly, they also recognized that DNAX scientists had made an

incredible reputation and inroads in the field of immunology. They were now looked upon as leaders of the field. And for an organization that had a bad image in the research field, DNAX was a jewel in their crown. I mean, they could boast that the number of papers published, number of symposium speakers, and by almost every criterion, DNAX was one of the leading research places in immunology.

DNAX had made several discoveries, which while they didn't have commercial rank, had radically transformed the field of immunology. I mean, the whole idea that there are two classes of T-helper cells, each secreting unique sets of cytokines was a bombshell. And to this day, DNAX is cited as the place where these discoveries occurred. Today, DNAX scientists are considered amongst the leading ones in the world.

Hughes: How did Schering-Plough commercialize this very prominent research group that they had on the West Coast? How did it help them sell their products?

Berg: Well, I don't think it helped them sell their products. There are two, I call them, intangibles.

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Berg: There's no question that today Schering-Plough in New Jersey is a first rank research organization, whereas when DNAX first joined them, it was pitiful. So there has been a transformation in their research organization.

Hughes: How did that happen?

Berg: Well, in large part because they recognized the disparity; that things coming from DNAX couldn't be implemented at Schering-Plough. People at Schering didn't fully understand them.

Hughes: So Schering began to attract better scientists?

Berg: Yes, with our help. We helped recruit people. We have been very active in Schering-Plough's activities, in recruiting leadership in various positions. They don't hire anybody in the science organization unless we approve it.

Hughes: Is that by contract or is that just an understanding?

Berg: I'll call it dependence. And recognition. Recognition of what we contribute. Nobody is hired at DNAX without each of us having interviewed them. I mean, Arthur, Charlie, and me. We've done a lot of active recruiting, that is, persuading people who are on

the fence about whether they want to go to academia or go to industry.

The second point is, DNAX had been incredibly efficient in generating new kinds of cell cultures, which then could be used for assaying special kinds of things. These were very valuable, and all of these were transferred to Schering. So Schering got technology, got materials, potential drugs, some of which are still in clinical trial, and a turnover in their own establishment. So, they've been transformed. And I think they rightly give DNAX a lot of credit for having catalyzed it, aided it. You could say, well, the way commercial companies judge value is not always the way we judge it. We think of it in terms of big, big accomplishments. But the transformation of a nearly moribund scientific organization on which a pharmaceutical company has to depend into one which is now recognized and can recruit very easily is an important accomplishment.

Second, their investment looks very good because, for not a lot of money, about $28 million at the time of the acquisition, they bought a world-class research organization. And they got a lot of--. Which word do I want?

Hughes: Kudos?

Berg: Yes. --for having done it. In fact, Arthur wrote this book [Golden Helix] because he was told that DNAX was used as a case study in business schools. People said, gee, somebody ought to write this all down.

Arrival at Stanford, 1959

Advance Preparation

Hughes: In our first interview, you said that you wanted to talk, and we did not, about your earliest experiences at Stanford. [interruption]

Berg: You have enough material about how we got recruited to come to Stanford.

Hughes: Yes, I agree.

Berg: So we had two years lead time to prepare for it, and that two years was one of active designing the building in which we were going to move, its interior wasn't yet designed, and preparing for what we hoped was going to be a totally novel way of teaching biochemistry. Remember that the group that moved here from Washington University were technically all microbiologists; we were in the microbiology department. We considered ourselves all biochemists, and in some ways we were parading under false pretenses.

Hughes: Standards.

Berg: The move here was looked to going to do something really novel and exciting in biochemistry. During those two years we helped develop a teaching program that was really remarkable and lasted for at least five to eight years in terms of being the most popular course at the medical school.

An Unfinished Science Building

Berg: In June of 1959, we were supposed to come here from St. Louis and move into the new building. When we got here, the building wasn't completed. We were told that it was on the way, and we should just wait. So we camped out, in a sense. We had a hut where the secretary sat who came with us. And we would come in every day and say, "Any news? Any news?" And, we would hear, "No, they haven't done anything."

So, eventually, we got so frustrated that Arthur, who had a lot of clout, went to the administration and said he was outraged, furious, and so on and so forth. And so what they did was gather all the workmen that were working all over the medical center, in the hospital and everyone out there, onto the third floor of the medical school building. And they finished us up in a very short period of time.

We had these moving vans that had transported all our lab equipment and supplies, and we moved right in. We moved in probably by sometime in July, and by the middle of August everybody was doing experiments.

Settling In

Hughes: And teaching?

Berg: We started teaching again in September. We had a new course. We were all teaching in new areas. But the research was going on. We had every barrel, every box marked, ticketed almost exactly to which drawer everything was supposed to go in. We had worked very hard to sterilize and wrap all the stuff we wanted to take. When we came, we had a carpenter; he built the dividers and drawers. It went from the barrel right into the drawer.

The only glitch we had was that there were a lot of air vents and things built in, and we couldn't get certain instruments onto the table because these things were sticking out. So we unscrewed them and capped them. But the building had never been officially tested. They put pressure on all the air lines or the gas lines to see that they don't leak. Well, of course, they leaked like a sieve because, what we had put in was not standard. [laughter] So we got the fire department down our back. There was a little flap.

Hughes: They knew that biochemists were in town. [laughter]

Berg: Yes, and working very quickly. It was really an amazing move.

Hughes: You had been orchestrating it for almost two years from St. Louis, from what you said.

Berg: Everything was planned down to a T. We brought with us secretaries, technicians, students, even shop people. And so we had everybody ready to transform the floor into a working unit.

The Stanford Department of Genetics

Lederberg's Arrival at Stanford

Hughes: I got the impression from going through the archive that a collaboration or a partnership, whatever you want to call it, with the Department of Genetics headed by Lederberg was really on people's minds. And yet, from what I can tell, it did not really materialize. Am I right?

Berg: Yes, to a certain extent. Josh Lederberg was in the field of genetics, probably one of the shining bright stars. He had been invited to come to Stanford and turned it down. But when Kornberg and we accepted to come, I think it might have been Kornberg who contacted him and said, "Think again. We're coming, and I think that between your genetics and our biochemistry, we can really be a powerhouse." And Josh changed his mind.

Now, unfortunately, when Josh changed his mind, there was no space, because there hadn't been any program to have a genetics department. So we gave him space in our department.

Hughes: There had been no genetics department?

Berg: There had been no genetics department. In fact, I think Stanford was the first medical school to actually create a genetics department.

Hughes: Is that so?

Berg: Yes, 1959.

Hughes: So, it was the Department of Genetics in the Department of Biochemistry?

Berg: Well, it was the Department of Genetics autonomous in every way. But the space that they used was in biochemistry space.

Stanley Cohen's Associations with the Biochemistry Department

Hughes: Because Genetics and Biochemistry were in more or less the same place, it was easy for people like Stan Cohen and Sgaramella to participate in biochemistry seminars. Is that right?

Berg: That's right. Only Stan never was really located in that space, because when Stan came to Stanford in 1968 he was in the Department of Medicine. He was recruited to become the director of the Division of Clinical Pharmacology. So he had space somewhere else.

I knew him before because he came from a lab of one of my closest friends, Jerry Hurwitz at Albert Einstein College of Medicine. I knew about Stan and when he came, I certainly had an open feeling for him. He then hit on plasmids as the thing to study in the area of drug resistance, which was clinical

pharmacology. When we found that the enzyme EcoR1 would make cohesive ends, and Mort Mandel discovered how to incorporate DNA into cells by giving them calcium shock. Stan started hanging out in Biochemistry because he saw that we were working now with plasmids. My student Janet Mertz was doing all these experiments, and she taught Stan how to do these transformations.

Hughes: So, up until then, he hadn't been around very much?

Berg: No, not at all. Walter Bodmer, who is now a distinguished professor of genetics at Oxford, interacted a good deal with our department. He and Josh formed the core of the genetics department. They had some postdocs, one of whom went on to become the head of the Eliza Hall Institute, Gus Nossal. Nossal, Bodmer and Leonard Hertzenberg were on our floor.

Lederberg and Space Biology

Berg: Josh was somewhat aloof and had very little to do with the research. In fact, at that time Josh was involved with planetary biology. He was learning about rockets and space biology and so on. So he really tuned out.

Hughes: Was that soon after he arrived?

Berg: Yes, because I remember, in 1961 I went to the International Congress of Biochemistry in Moscow. Josh was already actively involved in space biology because he enlisted me to sit in for him at this meeting in Moscow with other space biologists. I didn't know anything about it. But anyway, he was very heavy into it.

Josh told me, he denies it today, but I remember very clearly. He used to come walking by my lab on his way out, because his office was what became my office when I became chairman. He was on my corridor. He often would stop in with his arms full of books on planets, cosmology, astronomy, and rockets. He would come back the next morning with the same books already digested. He was remarkable.

Anyway, he said that he left what he had been doing before in genetics, in part because he realized-- The structure of DNA was published in 1953. From '53 to '59, Josh was skeptical of the notion that DNA could explain the gene's properties. He argued that genetics couldn't be explained by this molecule; there were too many complicating features about genetics that couldn't be explained by this simple molecular structure. Josh, I think, was

one of the last hold-outs, but by the time he came to Stanford, he was convince that it was correct. Nevertheless, I think Josh recognized that he was out of it because he was never molecularly oriented; he was largely a classical geneticist. And now genetics had become molecular, and he wasn't into it. Walter Bodmer, along with Ganesan, a student of Josh's, was doing transformation; Nossal was doing immunology, and Josh had to find something new. Space biology was sort of blossoming as a possibility, and he just went right for that. Josh says today that he doesn't remember ever saying that. But I remember very clearly because I was really astonished to see how somebody who was as extraordinary as he is eventually felt that he had been eclipsed.

Faculty and Tenor of the Genetics Department

Hughes: What about the other people in Genetics? Was there much interchange with Biochemistry?

Berg: No. There was Indian fellow named Ganesan. He was a graduate student, and he worked with Bodmer on DNA transformation. Josh brought Luca Cavalli-Sforza, who was a human geneticist, to Stanford. A wonderful man who had had a strong history in microbial genetics but now had gone into human genetics.

It was an interesting culture. Josh's department was very different than Biochemistry. Biochemistry was extremely interactive. Josh's department was, you're on your own. Everybody was on their own. There were very few faculty meetings. It was a non-department. Josh was involved in traveling around the globe doing his things. People were doing their own things.

Minimal Interaction between Biochemistry and Genetics

Berg: I think Arthur would probably say, and I would too, that a coalescence between Genetics and Biochemistry never really happened. It didn't happen in the teaching; it didn't happen in research. There was virtually no collaborations that were established. And there was no real intellectual interactions. We knew them and we knew what they were doing. I think the closest it came was when Stan started coming up to the department to interact, with Mort Mandel, who was a visitor in Dale Kaiser's lab, and with my graduate student, Janet Mertz.

Hughes: How about Sgaramella?

Berg: Sgaramella was also a postdoc in genetics. He had come from Khorana's lab. He came to my group meetings, and I'm sure he came to biochemistry department seminars, but I don't remember that as well. But I never saw him as a strong element or a strong part of the genetics department. He was a transient, who had already come with a problem. I mean, the problem that he worked on in Khorana's lab was this discovery that T4 ligase would join blunt-ended molecules. He certainly came to our group meetings and he participated in the discussions. I'm sure he must have given a presentation of his own work, but I don't remember it.

Biochemistry's Policy on Joint Appointments

Hughes: So it wasn't a rich exchange back and forth?

Berg: No. But you also have to remember that Biochemistry was somewhat aloof. Biochemistry itself was quite snooty; it didn't interact with anybody. It had a policy: it would not offer joint appointments to anybody, whereas lots of other places would help in the recruitment of a person for one department by giving them a joint appointment.

When I was chairman of the department [1969-1974], I sat on several committees that were trying to create a cancer center. The notion was that there would be some new building, and people would be in that building but have their appointments in various departments. I literally torpedoed those kinds of ideas because Biochemistry would never allow any of its faculty to be in other than Biochemistry space.

Hughes: As chairman, you could have led a crusade to change the policy.

Berg: Yes, but I believed in it. Actually, there was one other person in Genetics who was really terrific, and that was Eric Shooter. Eric Shooter came to our department as a postdoc; he was already a pretty senior guy. But he came on sabbatical to work with Baldwin. And then he went back to England. Then Josh got a gift from the Kennedy Foundation to create a neuroscience program. He recruited Eric Shooter to come back and head up this neuroscience center. So Eric was given a joint appointment in Biochemistry.

Hughes: He was the first in Biochemistry?

Berg: I can't remember. I think he was the first significant one. He was located in the genetics department. The genetics department and Biochemistry were separated by a swinging door. One of our graduate students elected to work with Eric Shooter. Within six months, he asked to be reassigned to somebody in Biochemistry. He found being isolated uncomfortable. He was mixed in with postdocs from Eric Shooter's lab, and they were doing genetics. He didn't feel part of the genetics department. I think that convinced most of us that those kinds of things don't work. If you have a faculty member in another building, and he has graduate students, those graduate students don't become part of the culture of the main department. So we resisted giving joint appointments. In fact, it was only about two years ago that we actually had the next such appointment, it was Gilbert Lhu, who had been a postdoc in my lab.

Hughes: Since 1959? That's amazing.

Berg: Well, actually, the Shooter joint appointment was probably in the mid-sixties.

Interdisciplinarity

UCSF

Hughes: I'm thinking of the very different model at UCSF, at least as it's been portrayed to me, in which departmental lines are quite porous. The Program in Biological Sciences [PIBS] is totally interdisciplinary.

Berg: But you're talking about now. That wasn't the way it was in the sixties.

Hughes: That's true.

Berg: PIBS was almost the last five years.

Hughes: Is it that recent?

Berg: That's right. Mike Bishop was one of the creators of PIBS.

Hughes: The idea of a much less departmentally oriented effort began to evolve at UCSF in the 1970s.

Berg: I would place it more in the eighties. I'll tell you why.

A Consolidated Stanford Graduate Admissions Policy in Biology

Berg: There was a sense that Stanford biochemistry as a department was beginning to compete for graduate students with places that offered more variety and opportunity for graduate students. While we had been extraordinarily successful in recruiting graduate students, we were beginning to lose out to places like MIT, UCSF, Caltech that were now creating larger entities into which students could enter and then select where they wanted to specialize. Whereas we had a very restricted set of options. We had only ten faculty.

Hughes: And you did biochemistry. [laughter]

Berg: That's right. And we didn't offer opportunities to somebody who said, "I'd like to come to Biochemistry but I want to do cell biology." So that was one of the impetuses for CMGM [Center for Molecular and Genetic Medicine]; that's the whole thing. I tried to get the department to agree that we would have a schoolwide admissions process, not identified as biochem. Until two years ago, Biochemistry said, "We don't want any part of any kind of bigger departmental thing. We'll only diminish the quality of the students we get. We'll have to compete with other departments. Other departments will either admit people that they think are good enough but they're not our type."

So I started a program for admissions in CMGM which was to run in parallel, because I knew there was no way that I could eliminate the biochemistry program or its admissions process. So I started our own. We started one under the center's auspices which brought in students and allowed them to remain undifferentiated, uncommitted for a year. They could go to any department in the medical school.

Hughes: Has that been successful?

Berg: It has now become the program under which our graduate students are admitted. Jim Spudich, who is the current chairman, finally began to recognize that in the program that Stanford had every department was going through their own process, reviewing applications. It made more sense to lump together all the "biology" at Stanford, offer a program that admits a student to Stanford biology, and then give them a year to decide where to do their Ph.D. research. Some go to biology, some go to biochemistry, some go to pharmacology, some go to genetics, and that's much more efficient. Last year, we got a terrific group of students.

The thing that was going on at UCSF was a very strong interest. As we began to learn of the development of the PIBS program, we could see that what was happening was we were having to compete with people who could offer students a much richer environment.

Beckman Center Programs

Hughes: These interdisciplinary programs also reflect what's happening in science. So you have to train people in an interdisciplinary way.

Berg: Yes, so at the Beckman Center, we retained the departmental structure, because I think it's the most efficient administrative unit. And probably for creating a kind of social structure that interacts and creates loyalties, it's good. Overlaying that, we have created a whole series of interdisciplinary, interdepartmental programs focused around themes.

We have one that is called Cell Sciences. So it brings people from Developmental Biology, Biochemistry, Pharmacology, Genetics. They all have an interest in the structure of a cell and how it's organized, what are the skeletal elements, how do things get shunted around. We have another one in immunology; again, it brought together people with very different backgrounds, including clinical departments, including people over on the campus--biology.

Now we have four such programs. We have one in human genetics, one in immunology, one in cell sciences. We are now in the midst of trying to create one called structural biology. Because, just for the reason you say, the problems have become so immense, that no one individual can bring to bear the kind of insights, the technical expertise, or the knowledge to be able to attack that problem in a comprehensive way. So what you have to do is bring people together.

The whole idea is to create groupings that meet together, talk together, get a grant together, have retreats. So I use funding that I get from the Beckman Foundation to seed these programs. And sometimes I have to do it by seduction, like the cell sciences program. People were saying we didn't have good microscopy, and cell biologists really need good up-to-date microscopy. I said, okay, I will create a superb microscopy facility, state of the art, if you guys will put together a program that will build the intellectual activities around it. So we have a terrific facility. We expend $100,000 a year to

maintain it, but people are now doing advanced kinds of microscopy that they've never done before. We have the same thing for molecular structure. We have twelve computers sitting in a facility which is now dedicated to teaching people to do molecular modeling. So you begin to get people who talk to each other.

So the department lines now have become what I like to call very permeable. People and ideas go across departments easily. But structurally it's still Biochemistry, still Genetics, and so on. It took a while to get my colleagues to accept that, Arthur being one of the most resistant to the--

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Berg: --Beckman Center concept. But I think today he has a very different view of it. I think he sees it as valuable.

Beckman Center for Molecular and Genetic Medicine

Origin of the Concept

Berg: To get to how the CMGM came about: During the recombinant DNA controversy, many of us who were participants were saying that to justify going ahead with something that might be slightly risky we need to consider all the benefits and rewards that would come from it. And most of those, we forecasted, were going to be in medicine. Some predicted it would be in agriculture, but most thought it was medicine that was going to be impacted the most.

I had a visit from a professor of medicine here, Kenneth Melman. He said, "You go around talking about all these things that are going to happen in medicine. I don't have anybody in my department who even understands the words. So if you think that we're going to translate these great things that you guys are doing into practical medical benefits, you have another guess coming. That was a sobering thought, because I always thought our Department of Medicine was pretty good. But they were of a different vintage. And the field was exploding.

So Ken Melman and I began to meet with the dean and we said what we really need to do is to create a new entity at Stanford which is composed of people who are trained in medicine but have elected to do science. Examples of such a breed were Mike Bishop, Harold Varmus, Mike Brown, Joe Goldstein. These are people who, following medical school, and clinical training, got intensive

basic science training and elected to follow a career in basic science instead of clinical medicine. But they always have in mind the nature of the biological problems, the medical problems.

One could foresee that if you got these people organized in some way, you could have very easy transfer of new information, new discoveries, new technologies, to the "clinic". In other words, you want to have a group of people who can speak to both physicians and the scientists and have the respect of both; I dubbed it the bench to the bedside activity.

We sold that bill to the medical school and the university. The idea was to create a department of eight new faculty, all to be newly recruited. We had identified a group of people, many of whom came out of M.D.-Ph.D. programs, who were looking for just this kind of an opportunity to bridge across the two fields. But there was no space to house them. There was zero space. The medical center was filled.

By the time we got around to thinking about building a building, we realized that we had other needs for space as well. For example, developmental biology was exploding. So why shouldn't we have a Department of Developmental Biology? We had a Department of Physiology which had become moribund, literally. Physiology was being taught to the medical students by physicians who weren't at the cutting edge. And so we argued that we should create a new kind of department which was dubbed Molecular and Cellular Physiology. The departments were Molecular and Developmental Biology, Molecular and Cellular Physiology and Molecular and Genetic Medicine. Those names were not accidental choices; they were all chosen to drive home that molecular was the level we wanted to understand things at.

Raising Funds

Berg: Well, once we decided to have three departments, it was clear we would need big money and a big building. The Howard Hughes Medical Institute was then headed by Don Fredrickson, who had been the former director of the NIH, and who on his own had been trying to promote this kind of training in people. Physicians who do science. When he heard that we were going to do that, Howard Hughes offered us $12.5 million towards creating this kind of a center. The Howard Hughes ultimately added another $7.5 million, for a total of $20 million. They adopted the Department of Molecular and Genetic Medicine and called it the Howard Hughes Institute Unit of Molecular and Genetic Medicine. HHMI doesn't

have departments. They also agreed to fund twelve investigators, plus the $20 million towards the construction.

Actually, I got a little of the chronology wrong. There was a steering committee created to try to develop this concept. Besides me there was the dean, Dominick Purpura, Stanley Cohen, Hugh McDevitt, and Ken Melmon, the head of medicine at that time. We brainstormed and focused on having a center for molecular and genetic medicine.

At that time, Arnold Beckman had let it be known that he wanted to give away his fortune before he died, and that he would entertain proposals for new projects. We put together a proposal; we invited him to come; he spent three days; we outlined our vision; he went away; we never heard from him again. We only found out later why.

In the interim, I proposed that we have an annual symposium called the Symposium on Molecular and Genetic Medicine. We couldn't build a building without money; we didn't have any people, but we ought to at least start promoting the term, the concept, on a national scale. So we had several terrific national symposia on this theme. Fredrickson came to one of them, and we told him our plans. He was going to become the president of the Howard Hughes Medical Institute and he would recommend investing $12.5 million in the CMGM and ultimately more. So we had a start on the funding. But no word from Beckman at all.

We had $12.5 million; the building was going to cost something like $40 million, and because we were so far short of the cost the trustees would not allow us to move ahead, either to plan or do anything. But Beckman remained silent. Only later did we learn that he was not about to fund a project under the aegis of a "directorate", our steering committee. He felt, "That's not the kind of organization I want to support. I want to see one person who has committed his energy, vision and activity full-time, and whom I believe and trust. I won't give money to something that is going to be run by a group." With no word from Beckman, the project was moribund. At that point Don Kennedy came to me and he said, "Either you become the director, or we drop the whole thing."

I had been asked several times to be the director. I had refused; I didn't want to do that. I was perfectly willing to spend time on this committee developing the concept. But I did not want to be the lead. But when Kennedy threatened to trash the whole project, I agreed.

Within weeks, I called Arnold Beckman and I said, "There has been a change in our plans. I have become the director. I'd like to come to talk to you about my vision for what will happen. And, he invited me to come. I asked Don and Arthur to join me. We met with Arnold Beckman for twenty minutes. And two weeks later he called and said he'd give us the twelve and a half million dollars we asked him for. Later, when the big announcement about his gift was made, he was asked why he was making this gift considering that he had no affiliation with Stanford. He said he knew me; he knew Arthur; and that he had complete confidence in us. If I was willing to take the responsibility of directing this whole effort, then that was good enough for him.

Hughes: Quite a compliment.

Berg: It was. So now we had thirty some odd million dollars, and then Arnold Beckman helped us raise some money from other sources, which he had access to. He persuaded SmithKline Beecham, then SmithKline Beckman, to give us $8 million dollars. It's rare that an industrial company gives you money to build a building. So we got the Center built.

Berg's Strategic Decisions

Berg: There were several strategic decisions which I had to make, which in retrospect I believe were the right ones. I should have said before: When I was asked to become director, I said I had two conditions. One was that the school would allow the building planning and construction to go ahead even if we didn't have all the money in hand. Kennedy agreed to that. And second, biochemistry had to become part of the center. I said there was no way I was going to devote the next five or ten years of my life and leave my colleagues sitting in the old building in antiquated space. Well, it wasn't quite antiquated, but they had to be part of the center. I also believed that there would be an enormous drawing card for recognition of the center if the famous biochemistry department was going to be in it. That meant the building had to be bigger and cost more money. The cost was going to be up around $50 to $60 million. So the job of fund raising came to be a big job. But we raised almost $96 million in those three years. I spent a lot of time on the road, meeting with people, persuading them. Dave Kern was very important and Arnold helped as well. But, eventually we got the money to go ahead.

Another strategic decision was the three new departments in that building, besides Biochemistry, were to be comprised of

people who were recruited from outside, not who were already ar Stanford in existing departments. There were two reasons for this decision: you could gather up all the stars that existed in Stanford departments and move them to the Center and leave behind a wasteland. That was the worst thing you could have done because it would have really created a lot of anger and anxieties. Those ransacked departments would now have had to go out and recruit replacements.

We had the advantage in being able to do that. We had a new building; we had a terrific concept. So I said we were not going to move any people. And Stan Cohen became my bitter enemy, in part because he was on that steering committee. I recall once when I came back from a trip to find that Stan had carved out space for himself in that building, as had Melmon and a few other people. When I came back I raised hell. When I became director, I said, "There are not going to be any moves." Obviously, I was vulnerable because I had insisted that Biochemistry would move to the Center.

Hughes: How did you handle that?

Berg: I said straight off that that was my price for being Director. I had made it clear that I was not going to do it without benefitting my colleagues who I was convinced would enhance the standing of the Center and thereby aid in the recruiting of new people. I think that was correct, indeed Arnold Beckman agreed.

Hughes: The other departments were created de novo?

Berg: Yes. So we set about recruiting. Eventually, we had to give in a little on the decision of nobody from Stanford. The building was going to take two and a half years to build. Howard Hughes had committed money and positions, and they kept saying to us, "Who are the Howard Hughes investigators?" Well, you couldn't recruit anybody to a building that was not going to be available for two and a half years. So we eventually identified a few people within the school who almost surely would have been Howard Hughes investigators anywhere else. The three of them were Jerry Crabtree, Irving Weissman and Gary Schodnick. That's all we did. They became the founders of the Howard Hughes unit, which ultimately reached twelve people. So everyone else was newly recruited--new chairmen, and new faculty.

To this day, I'm absolutely convinced my decision was right because had we filled the Center with the people we had, we never would have been able to create the kinds of connections back to the departments. The whole idea was to use the Beckman Center as the focus for this concept of molecular genetic medicine and build

bridges to existing people. So we recruited people who could relate to or connect to the strength we already had, and that happened.

The second important decision came from the recognition that there was a political problem. There were a lot of people who wanted to be in the Beckman Center, new space. We frequently heard, "I do molecular genetic medicine; why shouldn't I be in that building?" We had to admit, "We all do molecular genetic medicine."

The Program in Molecular and Genetic Medicine

Berg: It turns out there are a hundred and ninety people who do what could be called molecular and genetic medicine. We can't all be in one building. Instead, we can be part of and intellectual group called the Program in Molecular and Genetic Medicine [PMGM]. Members of PMGM differ one from another in where we live. In terms of funding, access to facilities, teaching opportunities, you name it, everybody has equal access, and that's what we have. In a sense the Program in Molecular and Genetic Medicine serves as an umbrella for those interested in molecular and genetic approaches to biological questions.

The physical focus is the Center. The Program encompasses people in the basic science departments, the clinical departments, chemistry, biology, applied physics. There are now 196 people who declare themselves as members of the program. They are invited to all kinds of PMGM functions. They've organized these interdisciplinary units on various themes. They have their own retreats seminar programs. We try constantly to meld basic science with clinical activities. We have workshops where we have brought physicians in to tell us about bone marrow transplants; we have immunologists and gene therapists who want to use bone marrow transplants.

It has been an exciting enterprise. When I retired from my professorship in 1998 the dean said, "I want you to remain as the director of Beckman." Actually, I don't direct anything other than to try to create new kinds of interactions. One has to be clever and imaginative to get people to come together when they're all busy doing their own thing.

Stanford Biochemistry's Asilomar Conferences

Berg: The Asilomar conference was my invention when I became chairman of the biochemistry department. We needed a way to get people in the department to talk to each other about their research. We used to do it in the department library but people were sneaking out to the lab or going home early. It was clear that the only way it would work was to go away from Stanford and its distractions. We started the Asilomar conferences in 1970 or '71, and they've been running ever since, not always at Asilomar. Now it's the highlight of the scientific year.

Hughes: Who goes?

Berg: Everybody; there were 175 people that went to the Stanford Center at Fallen Leaf Lake at this last one. I just got back last week. Every group is given a slot of time, usually 60-90 minutes, for presentations. The head of the research group programs what the people in his group will present. There is a lot of time for discussion. Those that don't get a chance to talk about their work, do it through poster sessions. Most people feel it's the best scientific meeting they've attended in the year, because there is terrific research going on. It lasts three days, and is intensive, nose-to-nose. People eat every meal together. That format has been copied all over the country. UCSF has started it; Berkeley has started it; Harvard, MIT. They all have retreats; they all have found local places where you can go and hide.

Hughes: Stanford's was the first?

Berg: We were the first.

Greatest Contribution

Hughes: I have one more question, but is there anything you want to say before that?

Berg: No.

Hughes: What do you consider to be your greatest contribution?

Berg: Some years ago there was a celebration of Severo Ochoa's seventieth birthday, in Spain, and all of his former students, colleagues and friends were invited to go. I think Arthur was one of the co-organizers. I was not a student of Ochoa; I had never

had any collaboration with Ochoa. But for some reason early on he took a liking to me, and he always treated me like one of his "children", so I was invited to go to Spain for this symposium.

We were each asked to write an essay. Most people just wrote a paper about some of their recent work. I tried to do something different. I tried to ask what is the measure of somebody's greatness or the contribution they made. What meant the most to me is the long-term impact of ones work or efforts. Pebbles dropped in water produce a succession of ripples, and one might ask what those ripples created. Is it just the impact of the stone, or is it the effect of the ripples that's important? I tried to think of it in terms of who have these people trained? Who are the people who have brought their style, their message, their philosophy, their whole concept of science and taught their students that way.

I had the idea of trying to measure this by way of a star chart. An individual would be represented as a dot. The people that person trained would be represented by radiating lines from that dot. The length of the line was to be proportional to the impact or success that person had achieved; that would be a measure of his or her accomplishment. Then those people would have trained somebody, and there would have been branches off that line. You would have created a halo around this dot. The density of the halo would be a measure of what the person had contributed to science, and how far the halo came would be some measure of how successful his progeny were in science.

I tried to do that kind of representation. Ochoa was one, and I was going to have about five or six other heroes in science to get a measure of their impact. Some of them have done great science and got the Nobel Prize but hardly ever trained anybody, or ended up with flunkies who never went on to do anything else. I wanted to be able to illustrate that, but Arthur talked me out of doing it. He said, "You will make enemies that will last a long time. How are you going to be able to identify who this line is, and who the short stub is?" I thought better of it, and I didn't do it. But the concept has always stuck in my mind.

When you ask me what has been my greatest contribution, I think it's the students that have come out my lab, and the tremendous science that many of them have done, and the affection ad relationship that exist between us. I'm still in touch with so many of these students. They put on a party for me on my sixty-fifth birthday, which would blow you away. The party took over the Mark Hopkins Hotel, and the DeYoung Museum. The museum has a magnificent hall hung with tapestries, which served for the dinner. The organizers, all students, raised $160,000 for this

party. Former students and postdocs came from Europe, Asia, all over the world. It was an incredible party. Anyway, that relationship and admiring what they've accomplished to me is the most important thing.

Many of them say that the way we did science in the lab at Stanford has always influenced the way they do science and what they try to convey to their students. To me, that is probably more than just the few things that I've done, or done with them, because that radiating effect is going to influence much more of science than I could have done alone.

I think about our department in the same way. Our department has done a lot of great science; It's not possible to talk about contemporary science without running into someone who was a postdoc or student at Stanford. That's a very satisfying feeling.

Hughes: Thank you.

Transcribed by Quandra McGrue
Final Typed by Grace Robinson

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SAMPLE EDITED PAGE
(from transcript, with narrator's editing)

molecule, and inside the cell, it ~~immediately~~ circularizes and the nicks ~~or spaces~~ between the ends get closed. And ~~now~~ it functions as a circular DNA molecule. ~~Okay?~~

Creating Artificial Cohesive Ends

Berg: So, the concept of sticky ends ~~was~~ already ~~there~~. If you want to join two different molecules together, it doesn't take ~~a genius~~ genius to figure out that if you can ~~actually~~ create artificial ends that are complementary to each other ~~then~~ the two will come together. Right? No big deal.

So, ~~the question is, if you have a piece of DNA A and you have SV40 DNA B~~, if you put tails of A on ~~one~~ and tails of T on ~~the other~~, and ~~you~~ mix them, the A's and T's will form double helices, and the two molecules will come together. ~~This~~ cannot join to itself. ~~You~~ could have used G's and C's, but A's and T's were easier to add.

We already knew how to add tails onto ~~these~~ DNA molecules because there is an enzyme that had been described which is present in ~~the~~ calf thymus, and has an interesting physiological function, but ~~it~~ was not known at ~~that~~ time. It ~~was~~ DNA polymerase, but ~~it's~~ a "dumb" DNA polymerase. It doesn't need a template. If you give it any one of the four deoxynucleotides, it will add ~~it~~ on to the end of the DNA molecule ~~and on and on~~. So, if ~~you have your~~ DNA molecule A, ~~and you put in~~ deoxyATP and this enzyme, ~~it will polymerize~~ A's onto the two 3-prime ends of this DNA. And if you do it with deoxyTTP ~~with this DNA molecule, it will put~~ T's on. By regulating the time of the reaction, you ~~put~~

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Stanford University School of Medicine
Stanford, California 94305

Born: June 30, 1926, New York, New York
Address: 838 Santa Fe, Stanford. CA 94305
Soc. Sec. #: 095-18-1653

Education:

1948 B.S., Pennsylvania State University
1952 Ph.D., Western Reserve University

Professional Background:

1950-52 Predoctoral Research Fellow, National Institutes of Health.
1952-54 Postdoctoral Research Fellow, American Cancer Society, Dr. H. M. Kalckar, Institute of Cytophysiology, Copenhagen, Denmark and Dr. Arthur Kornberg, Washington University School of Medicine, St. Louis, MO
1954 Scholar in Cancer Research, American Cancer Society, Department of Microbiology, Washington University School of Medicine.
1955-59 Assistant to Associate Professor of Microbiology, Washington University School of Medicine
1959-60 Associate Professor of Biochemistry, Stanford University School of Medicine
1960 Professor, Department of Biochemistry, Stanford University School of Medicine.
1969-74 Chairman, Department of Biochemistry, Stanford University School of Medicine
1970-94 Sam, Lulu and Jack Willson Professor of Biochemistry.
1973-83 Non-Resident Fellow of Salk Institute.
1985 Director, Beckman Center for Molecular and Genetic Medicine.
1994 Named Vivian K. and Robert W. Cahill Professor in Cancer Research

Honors:

Eli Lilly Award in Biochemistry (1959)
California Scientist of the Year (1963)
National Academy of Sciences (1966)
American Academy of Arts and Sciences (1966)
Henry J. Kaiser Award for Excellence in Teaching at Stanford University School of Medicine (1969, 1972)
Distinguished Alumnus Award, Pennsylvania State University
V.D. Mattia Prize of the Roche Institute for Molecular Biology
Institute of Medicine, National Academy of Science (1974)
President, American Society of Biological Chemists (1975)
Honorary Doctor of Science, University of Rochester and Yale (1978)
Sarasota Medical Awards for Achievement and Excellence (1979)
Annual Award of the Gairdner Foundation (1980)
Nobel Award in Chemistry (1980)
Albert Lasker Basic Medical Research Award (1980)
New York Academy of Sciences Award (1980)
Foreign Member, French Academy of Sciences (1981)
American Association for the Advancement of Science Scientific Freedom and Responsibility Award (1982)

National Medal of Science (1983)
American Philosophical Society (1983)
Associate Member of EMBO (1984)
Honorary Doctor of Science, Washington University, St. Louis (1986)
National Library of Medicine Medal (1986)
American Academy of Achievement (1988)
Honorary Doctor of Science, Oregon State University (1989)
Special Achievement Award, Odyssey Biomedical Corporation
Fellow of American Association for the Advancement of Science (1991)
Honorary Member of the Academy of Natural Sciences of the Russian Federal Republic (1991)
Foreign Member of the Royal Society (1992)
Fellow, American Academy of Microbiology (1992)
Honorary Member Alpha Omega Alpha Honor Medical Society (1992)
Honorary Member AMBO/AMBL (1994)
Honorary Doctor of Science, Pennsylvania State University (1995).
Member, Pontifical Academy of Sciences (1996)

Special Appointments:

Editor, Biochemical and Biophysical Research Communications
Member, NIH Study Section on Physiological Chemistry
Member, Journal of Molecular Biology Editorial Board
Member, Board of Scientific Advisors of Jane Coffin Childs Foundation for Medical Research
Member, Advisory Boards to National Institutes of Health, American Cancer Society, National Science Foundation, Massachusetts Institute of Technology, and Harvard University
Elected to the Council of National Academy of Science and to the Scientific Advisory Board of the Welch Foundation
Member and Chairman, International Advisory Board of the Basel Institute of Immunology
Chairman, Whitehead Institute Board of Advisory Scientists
Chairman, National Advisory Committee, Human Genome Project
Trustee, Rockefeller University
Chairman, Board of Directors, National Foundation for Biomedical Research
Chairman, Public Policy Committee, American Society for Cell Biology
Advisory Editorial Board, Molecular Medicine Today
Advisory Panel, Human Genome Education Program
Whitehead Institute Board of Associates
Scientific Advisory Committee, Research!America
International Scientific Advisory Board (ISAB)
Chairman, Scientific Advisory Board, Beckman Foundation
Advisory Board, McGovern Institute for Brain Research

Commercial and Civic Activities

Founder and Principal Scientific Advisor, Schering-Plough's DNAX Research Institute
Director, Affymetrix
Consultant, Bay Area Bioscience Center
Consultant, Santa Clara County Biotechnology Education Partnership
Council of Advisors to San Francisco Unified School District
Advisory Board, ARISE (American Renaissance in Science Education)

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Paul Berg

Application #10170 [1969]

We are trying to learn how viral genes can alter the growth properties of a cell and make it cancerous. An important goal of our research is to learn how genetic information carried by a virus chromosome can be integrated into the genetic machinery of a mammalian cell and thereby influence the properties of that cell. To study this we are examining a mutant virus which is unable to transform cells into tumor cells under certain conditions. We hope to define the defective function and to determine its role in the integration process. In practical terms, if we could understand how new genetic information can be inserted and maintained in a foreign chromosome, we might not only be closer to the secret of cancer, but also on the road to learning how to modify the genetic constitution of cells by integration of other types of DNA molecules.

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[1977]

Status of Preliminary Planning of Basic Sciences Sub-Committee of Cancer Center Planning Group

A strong Basic Sciences Program is an essential element of any effective Cancer Research effort. Without the continual infusion of basic science techniques, knowledge, and ideas, the momentum and inspiration for the needed research developments on the Cancer Problem would soon founder and dry up. With this premise, and indeed strongly held conviction, the Basic Sciences Sub-Committee of the Cancer Center Planning Group has conducted its preliminary planning by considering the following three general questions:

1) Stanford's present strengths in those areas of Basic Sciences Research which are relevant or bear directly on the Cancer Problem.

2) Stanford's needs for expanding, and strengthening existing programs as well as development of new programs in various areas of Cancer-related Basic Sciences Research.

3) Ways in which the Cancer Center's Basic Sciences Research Activities should be organized so as to enhance the Center's Clinical efforts as well as be consistent with Medical School's existing Research and Teaching Programs.

No definitive conclusions have been reached yet on any of these three problems but the following outline attempts to summarize some of our thinking in these areas. For clarity and brevity, the first two questions have been considered together under each heading.

1) Present Strengths and Activities in Basic Sciences Research Relevant to the Cancer Problem.

A) Molecular and Regulatory Biology of Animal and Human Cells:

Stanford's emphasis in this area of research has dealt mainly with bacterial cells and their viruses but more recently there has been an expansion of research with eukaryote systems. In spite of Stanford's acknowledged and recognized leadership in this field an objective review of these activities shows that it is the intensiveness rather than extensiveness that is our strength.

i) Basic Mechanisms and Structures Concerned with Replication, Transcription, and Translation.

Perhaps strongest is research centered about the enzymatic synthesis of nucleic acids, and in particular in prokaryote systems. Dr. Arthur Kornberg (Biochemistry) is continuing his elegant studies on enzymes involved in DNA synthesis. Dr. I.R. Lehman (Biochemistry) has been concerned for many years with various enzymes concerned with DNA

metabolism, and is currently studying the enzymatic mechanisms of genetic recombination in E. coli. Dr. A.D. Kaiser (Biochemistry) has worked at a slightly different level of DNA synthesis in studying the processes whereby DNA is synthesized and packaged into a completed virus particle, studies that have combined genetic, enzymatic, and electron microscopic approaches. Dr. David Hogness (Biochemistry), whose previous work had been on the genetics of lambda phage, has focused his undivided attention in the last two years on the molecular structure, regulation and expression (developmental) of polytene chromosomes of Drosophila. Dr. A. Ganasan (Genetics) has been studying the process of DNA replication in prokaryotes, and more recently in animal cells, centering his studies on the potential role of nuclear membrane-chromosome complexes in DNA replication. Dr. David Clayton (Pathology) is concerned with the synthesis and function of complex forms of DNA associated with mitochondria of malignant cells. Dr. Paul Berg (Biochemistry) has made important discoveries concerning the structure and function of RNA polymerase, the mechanism and regulation of amino acid activation and protein synthesis in prokaryotes. Not directly associated with the medical school, but an active intellectual input to the work going on here is Dr. Charles Yanofsky's group (Biological Sciences), whose studies on the regulation of the tryptophan operon in E. coli has been pioneering and one of the most germinal in the field of regulatory biology. Dr. R. Schimke (Pharmacology) has been concerned for a number of years with the mechanism and regulation for continual synthesis and degradation of cellular proteins of animal cells. Dr. Oleg Jardetzky (Pharmacology) is studying dynamics of protein folding and molecular interactions of DNA and regulatory proteins, specifically the lac repressor, by various physical techniques, including NMR spectroscopy.

ii) Regulatory Processes-including Developmental, Environmental, and Hormonal Influences

Dr. Arthur Kornberg (Biochemistry) has been studying the simplified developmental system of sporulation in B. subtilis, a process that involves degradation of cytoplasmic proteins, and the formation of an essentially new cell type, the spore. A number of individuals at Stanford Medical School are concerned with various aspects of regulatory processes, particularly those that concern developmental and hormonal-controlled processes. Dr. R. Schimke (Pharmacology) is investigating steroid hormone regulation of cytodifferentiation and function of chick oviduct with the eventual goal of isolating all regulatory components concerned with specific protein synthesis, i.e. genes, mRNA , protein synthesis factors, etc. and analyzing their response and behavior with respect to developmental and hormonal stimuli. Dr. Aronow (Pharmacology) has been examining the effects of corticosteroids on animal cells in culture with a view to defining the mechanism of that control system. Dr. Merton Bernfield (Pediatrics) is also interested in hormonal regulation of development, and is specifically studying the role of alterations in isoaccepting tRNA species in the regulation of specific protein synthesis in chicken liver. Another aspect of his studies is related to the role of epithelial-mexenchymal

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interactions in control of differentiation. Dr. Lawrence Kedes (Medicine) is studying the regulation of histone synthesis in the developing sea urchin, and in particular is utilizing techniques to isolate labeled mRNA for histone biosynthesis, to map the histone genes on the chromosome, and to study the activation of these genes during early embryogenesis. Dr. Frank Stockdale (Medicine) actively studies the hormonal regulation of mammary gland development, and the molecular events involved in myogenesis. Dr. Stanley Cohen (Medicine) has made important contributions to our understanding of the mechanism of development of resistance to a number of antibiotics by acquisition of drug resistance factors. He, as well as Dr. Korn's laboratory (Pathology), have made detailed investigations of the regulation of episomes, replication and transfer and its bearing on drug resistance and other pharotypic properties. Dr. Eric Shooter's group has had a strong program on analyzing the structure and function of the nerve growth factor; (NGF); NGF is a protein of animal origin which specifically stimulates outgrowth of fibers from embryanic sympathetic ganglia.

An Institute program in cancer biology will need considerable development in a number of areas related to macromolecular structure and synthesis. There is particular need for a group of investigators who are capable of studying the molecular structure of proteins and nucleic acids, including X-ray crystallography and sequencing of both proteins and nucleic acids. Facilities and expertise for these approaches, so fundamental to an understanding of regulatory processes, is totally lacking at Stanford. Other areas to be developed include the general area of regulation of DNA replication in animal cells. Programs should also be developed in the area of the structure and function of animal chromosomes, including the methodology for isolation of specific chromosomes and the analysis of DNA-protein interactions by various physical and biochemical techniques.

Another area of research which is an integral part of a cancer biology program and should be expanded at Stanford concerns the structure and function of intracellular organelles.

CELL ORGANELLES

I. Recent advances in the biology of mammalian cells have shown that is is quite feasible to study the various organelles of the malignant cell as separate entities. In particular, cell membranes, microfilaments and mitochondria have received increasing attention in the last few years. From these investigations it is clear that such systems are fruitful areas to pursue fundamental problems in mammalian cell Biology.

The specific areas currently under investigation by existing Stanford faculty are as follows:

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genome in human tumor cell populations taken at biopsy. This approach ties in closely with the proposed Human Cancer Cell Bank described in section E. In Hayflick's program human diploid cells will be used to determine whether several different oncogenic viruses including feline and murine sacoma and leukaemia viruses can, under special conditions, transform these normal human cells to cells having cancer properties. These special conditions include:

a) Extremely low virus multiplicities, coupled with the addition of specific immune serum.
b) Transformation of human diploid cells will be attempted utilizing a combination of very low level radiation and addition of low multiplicities of several oncogenic RNA viruses at several times post-irradiation.
c) Transformation of human diploid cells are being attempted using very low multiplicities of several different human viruses plus reduced temperature of incubation.
d) Attempts to transform normal human cells with chemical carcinogens as a means of comparing cell susceptibilities to viral chemical carcinogenesis; i.e. the compounds of 3 methylcholanthrene and benzo (a)-pyrene.

3) Dr. Paul Berg's group (Biochemistry) has an expanding and extensive research program on the Molecular Biology of the DNA Tumor Viruses - Polyoma (PY) SV40 and adeno viruses. Their work is proceeding along the following lines:

a) Identification of viral genetic functions for both lytic growth and cellular transformation leading to oncogenic potential in animals.
b) The mechanism and regulation of expression of these genes particularly the interplay of the host cell's and viral regulatory systems on each other; i.e. how viral genes perturb cell's growth regulatory machinery and how cell's regulatory systems affect readout of viral genes.

c) Identification of viral transcripts and the protein products produced in infected and transferred cells.

d) In vitro analysis of virus-directed processes in sub-cellular systems; i.e. nuclear and enzyme preparations.

e) Development of a system for viral mediated transduction of genetic information from one cell to another.

4) Recently Dr. George Stark (Biochemistry) has begun a program to isolate, characterize and define the function of the T-antigen produced in cells transformed and carcinogized by these PY ad'SV40 viruses.

5) Dr. William Robinson's program (Medicine) is directed at the Molecular Biology of the RNA Tumor Viruses particularly the avian group. These are the best known and so far most intensively studied. Dr. Robinson has already made numerous important contributions to our knowledge of the structure of the viral RNA genome, of the GSA and the Type specific envelope antigens. He is currently studying the synthesis of these viral-specific components in transformed cells as well as the nature and activity of the newly discovered RNA-dependent DNA polymerase. Quite clearly this system has been germinal in directing attention to the possibility that the RNA tumor viruses may well be important for understanding human cancers. Certainly many of the observations with the avian system has had direct relevance to the C-type viruses of other species.

It's quite clear that there is significant activity now going on at Stanford in this field of research. What is needed is an expansion of these activities particularly for increased efforts on genetic approaches to the RNA Tumor Viruses and for work with other mammalian RNA Tumor Viruses (feline, murine, etc.) Even more gaping in our inadequacy is work with other animal viruses. There presently is no substantial work with pox viruses of the reovirus type. All of these provide fertilization for work on the frank tumor viruses. We have little or no ongoing work with Herpes virus yet this class has recently been implicated in naturally occurring human as well as animal cancer. As pointed out in another section,

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ARTMENT OF BIOCHEMISTRY

PAUL BERG

Jack, Lulu and Sam Willson
Professor of Biochemistry

February 29, 1972

Dr. Michael Stoker
Imperial Cancer Research Fund
P.O. Box 123
Lincoln's Inn Fields
London, W.C. 2, England

Dear Michael:

So often in the last few weeks I've wanted to write to you but an avalanche of deadlines, from which I am only recently recovering, forced delay after delay. How are things at ICRF? Hopefully, science, construction progress on the new labs as well as the many administrative activities you are involved in are going well; Peter Beard's occasional correspondence with people in the lab and Bill Folk's visit brought only incomplete accounts of the happenings in London. Right now I'm wondering how you're faring under strictures of the fuel-electricity shortage; sounds pretty grim! Has it caused hardships in the labs, particularly with being able to maintain necessary services for work and keeping cells viable and alive? I suspect that long ago you had seen to ensuring the laboratories own power supplies and therefore immunity from external exigencies. From what I read in our newspapers I suspect you probably had trouble commuting from Kent.

In the last few months things have gone quite well here in the lab. Let me fill you in on a few of the details.

1) We've mastered the technique of detecting polyoma or SV40 viral DNA sequences by following the kinetics of annealing using S_1, an enzyme which rapidly degrades single-stranded DNA but leaves double-stranded DNA segments intact. In a nutshell, (I assume Bill Folk can relay more specific details of the procedure since he followed our protocols to the letter), we synthesize very highly P^{32}-labeled PY or SV40 DNA using pure E. coli DNA polymerase highly purified unlabeled Form I DNA as template, four very hot d-triphosphates (10-15 mc/μmole) and traces of DNAase to generate random single-strand nicks in the supercoils. DNA polymerase replicates random segments of the DNA (by "nick-translation") thereby generating highly-labeled viral DNA segments. The labeled DNA which has an average single-strand length of 300-500 bases and a specific activity of $5 - 10 \times 10^6$ cpm/μg is used as the "probe" for detecting complementary sequences in cellular DNA's. The experiment is quite simple now; e.g. P^{32}-PY DNA is denatured in the presence of either salmon-sperm DNA, BHK DNA or the DNA from cells abortively or stably transformed by PY. Salt is added to the appropriate concentration (0.2-1.5M depending on the rate of annealing we expect) and annealing occurs at 68°. Samples are periodically withdrawn, diluted and frozen. When enough samples have been taken, they are all digested briefly with S_1 and then precipitated with TCA. The amount of annealed DNA is equal to the amount of P^{32}-label precipitated by TCA and retained on the filter. At zero time this 2-4% of the input at the end of the annealin

70-80% of the DNA is insensitive to S_1, (the remainder is probably the fraction of DNA nucleotides which cannot enter into helical structure because of steric-hindrance, or we believe more likely, the non-viral DNA sequences carried by our polyoma stock). The protocol, computer printouts of the data and calculati as well as the photographs show a typical reconstruction experiment when know quantities of cold PY DNA (sheared to same size and denatured) is added to the annealing mixture. Recently, we've simplified this procedure so that samples are taken only during the first few hours of annealing and our computer progra computes the second-order rate constant (and thereby Cot 1/2) from the initial points. The second set of computer printout and plots use only the data for the first 24 hours and you can see that the curves are linear (2nd order) even with the points for the first two hours. The sensitivity seems to be good enough to detect 0.5-1 genome equivalent per cell (< 1 part in 10^6) using approximately 1.5 to 2.5 mg of cell DNA.

Using this method we've begun to analyze some of the abortive and stabl transformants of BHK we collected when I was in London. Summarizing:

a) P^{32}-PY DNA in the presence of salmon sperm or BHK DNA anneals with an identical Cot 1/2: Martin claims that 3T3 does contain some sequences homologous to SV40 but we shall have to do this many times before one can say definitely that there is or is not a fraction of a PY genome equivalent in normal BHK cells.

b) The abortives MA-8 (methocell) and SA-10 (surface-infected) have no detectable PY sequences. Of the two stables, transformants we've tested, ST- and MT-1, only MT-1 contains PY- DNA sequences. (MT-1 3-5 viral equivalents ST-1 < 1 viral equivalent/cell). Consequently, ST-1 may be a spontaneous transformant but it should be retested. If you have another sample of ST-1 with one that can be screen hopefully, one that could be tested for T-ag, we'd like to retest it.

c) We have just finished growing MA-4, MA-6 and MA-9 and SA-2 as well as one other stable transformant and will do the annealing kinetics with their DNA's shortly.

In view of Smith et al. finding with the SV-3T3 abortives it really is important to determine for sure if PY-BHK abortives really do not have PY-DNA sequences in their DNA. If there is a difference it could be very relevant to the mechanism of integration-excision with each virus. It seems crucial therefore, that we look at as many true abortives as possible to be sure. I hav only some of the clones we picked. Do you have more? Or even abortives coll in different experiments over the years? Or do we have to do another experim collect a new set (clones grown from microcolonies picked from methocell wou seem to be the best since they are most likely abortive). I'd be delighted to come over again to prepare a fresh lot but that's not possible now. Some time in September (Renato has been trying to talk me into coming when he's there in Sept.) would be possible but that seems a long way off; until them is it possible to get any abortively transformed clones, (either ones we collected in 1970 or

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any others you have) to look at? Accumulating enough cells for DNA isolation is a limiting factor since our large-scale growing facilities are still primitive. Is it possible for your set-up to produce about 5 gms wet weight of some of the clones? If we could grow some and you could grow others that might speed things up somewhat. Unfortunately, although we are in good shape to do the hybridizations easily enough we are in worse shape to generate and grow well characterized abortives.

2) Our project to insert new segments of DNA into the SV40 DNA molecu has succeeded and now we hope to get to some interesting experiments. Without going into detail here, unique SV40 linear DNA molecules (made by a single double-stranded break with a bacterial restriction enzyme coded for by the drug-resistance transfer factor RTF-1) have been derivatized at their 3'OH end with either short runs of dA or dT and after annealing to produce non-covalent dimer circles they were covalently joined with DNA polymerase and DNA ligase to produce sealed dimers in 25-35% yield. Using the same kind of SV40 DNA linears and appropriately derivitized linear DNA from λdvgal (mol. wt. 6×10^6 containing about 4×10^6 daltons of λ DNA and about 2×10^6 daltons of E. coli DN including the entire gal operon), covalently joined hybrid DNA molecules have been made in about 20-25% yield. Still to be completed (this has run into more difficulties than I anticipated) is the insertion of a short piece of synthetic DNA, dI, G:dC (100 base pairs), into the SV40 ring at the R_1 restriction site and ultimately at other sites in the structure. Hopefully, such molecules may give us a way to map the genes of SV40.

There are several other projects moving well enough (Peter Beard's work is coming along quite nicely) but more about these at some future occasion either here, in London, or at least by mail.

I do look forward to your comments on the question of the abortives and of course to hearing something about your own work on the serum factors, as well as of other happenings from ICRF.

My very best wishes and regards for the coming year to your family, and colleagues at ICRF. Please remember me to all.

Sincerely,

PB/1

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STANFORD UNIVERSITY MEDICAL CENTER
STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOCHEMISTRY

PAUL BERG
Jack, Lulu and Sam Willso
Professor of Biochemistry

March 28, 1972

Dr. Michael Stoker
Imperial Cancer Research Fund Laboratories
P.O. Box 123
Lincoln's Inn Fields
London, England

Dear Michael:

Your secretary wrote me of your visit to the States; I hope that you feel welcome enough at Stanford to let me know when you're within striking distance of Palo Alto so that we can arrange for a visit. We've come a long way since your last visit but more importantly we'd love to see you again.

It's really marvelous that you're able to resurrect more of the abortives and stables and to send us cells for the DNA preps. We have trypsinized the cells, quenched with serum and washed 1 time by centrifugation with Tris-saline and then frozen the cell pellets. If we got the cells you indicated that way it would be great. With respect to ST-1, we are repeating the determination with another batch of DNA and we'll do it with your cells as well; that should give a pretty definitive answer. As you say, if they remain transformed without having the viral DNA that would be interesting. Do you have anti PY-T serum? It could be important to know if ST-1 is T-ag$^+$ or T-ag$^-$.

I spoke to Helene Smith last week and she passed on some additional information. The hybridizable SV40 DNA in one of their isolates sediments with the cell DNA in an alkaline sucrose gradient done according to Sambrook et al. She can detect "no" infectious SV40 DNA in such cells. Presumably then it is "integrated". But in one of their clones, the one which gave variable amounts of SV40 sequences in different clonal isolates of the original abortive, and on different trials, contained no SV40 DNA after growing up a large batch of cells. Very strange indeed. The abortives that contain DNA show about 1% of the transforming efficiency of normal 3T3 with wild-type SV40 DNA virus. Didn't you find that BHK abortives could be transformed at normal efficiencies when challenged with PY?

We plan to make highly labeled P^{32}-SV40 DNA for use in detecting SV40 sequences. The procedure would be the same as for PY; purified SV40 Form I is used as template instead of PY I in the DNA polymerase reaction and that generates that reagent for annealing. We could test Warner's clones when they're ready.

We've just had another set of interesting results you might like to

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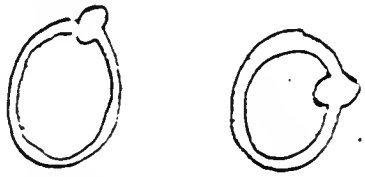
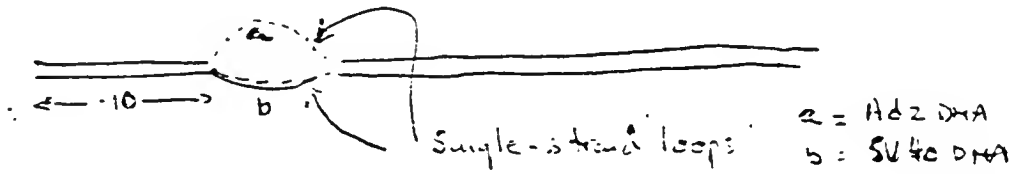
Dr. M. Stoker
Page Two
March 28, 1972

hear. One of my students, John Morrow , has been studying the action of the restriction enzyme, R_I (coded for by the Resistance Transfer Factor I, RTF_I, carried by E. coli) on SV40 DNA. This enzyme has been purified by Herb Boyer in SF who gave us some for these experiments. R_I cleaves SV40 Form I DNA (prepared from plaque purified virus) quantitatively to unit length linear molecules; the distribution of lengths (as seen in EM) is within 2% that of the circles. Each single strand is unit length in alkali so quite clearly the enzyme makes only a double-strand cleavage and no more. The molecules are unique; when denatured and then renatured they produce only linear molecules with the same length distribution as the starting molecules. Had they been circularly permuted linears or even two or several types such denaturation-renaturation produces circular molecules (the result found with linears produced by P_I or B-restriction enzymes or with DNA'ase). Further proof of the uniqueness of the break is the following: Delius, at CSH, has found that T_4 phage gene 32 protein binds to SV40 DNA Form I and if fixed to the DNA with glutaraldehyde and then spread on grids for EM inspection one obtains molecules with a single " bubble."

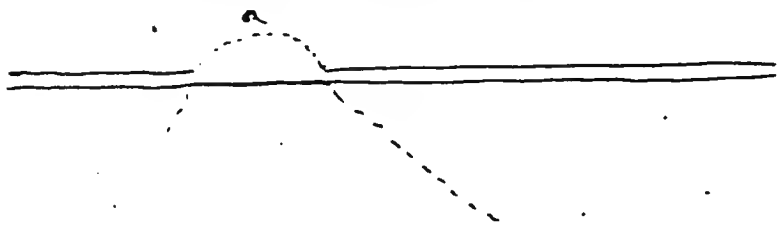


If such molecules are treated with R_I one obtains linear molecules with a bubble at only one location;

about 40% in from one end. Thus we can differentiate one end from the other. Most recently, John has done the following; if Ad_2-SV40 ND_1, the non-defectiv hybrid of Ad 2 and SV40 DNA (which, according to Lewis contains the "U-ag gene" of SV40 covalently integrated into the Ad 2 chromosome) is denatured and renatured with wild-type Ad 2 DNA one can observe the following heteroduplex in the EM.



There are two single-stranded (indicated as dashed lines) loops about 16% in from one end; one segment (a) is longer than the other. If SV40 R_I produced linears are included in the denaturation-renaturation mixture and the product is spread on grids one sees another picture:



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The shorter segment of the loop now appears double stranded and from it comes two single-stranded tails. The length of the tails and the paired loop is about equal to an SV40 length. Thus the R_I restriction enzyme makes a break near the U-ag gene. There is some reason to believe from other Ad 2-ND viral DNA's that the TSTA and T-ag genes are on the longer arm of the SV40 segment.

I'd like very much to come to London for a bit in September . If you're going to be there during the first three weeks or so it would be fun to come over and try to do some experiments. Will you be moved into the new labs then or is that just about the time you'll be moving in? I'll keep in touch with you about how things are going on this and other projects. If you know of any appropriate conferences or meetings in England or on the continent in the Fall, please let me know.

So much for now. My best to all at ICRF and to Veronica. Peter Beard is doing very well and I'm delighted at his being here. He's made several very interesting findings that are proving very useful; he sends his regards to all.

Sincerely,

Paul

PB/l

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EPARTMENT OF BIOCHEMISTRY

PAUL BERG
Jack, Lulu and Sam Willson
Professor of Biochemistry

June 19, 1972

Dr. Michael Stoker
Imperial Cancer Research Fund
P.O. Box 123
Lincoln's Inn Fields
London, W.C. 2 England

Dear Mike,

Another progress report! Marianne has, I believe, already let you know that the cells arrived in good shape and she will work them up soon to isolate the DNA's for testing. Thanks for making that part of the experiment easier.

Meanwhile we have run through a few more clones and repeated the annealing kinetics with the older ones. The results are quite consistent and give the same result (Table I); none of the abortively transformed BHK have any trace of PY DNA! In Cot #17 the $Cot_{1/2}$ would have decreased by a 2-fold if the cell DNA had contained 2.5ng of viral DNA; assuming the diploid cell genome size is $\sim 3 \times 10^{12}$ daltons of DNA, halving of the $Cot_{1/2}$ would have indicated 1 viral genome/cell. In Cot #20 we increased the sensitivity so that 1.2ng of viral DNA in 2.5mg of cell DNA would have halved the $Cot_{1/2}$. As you can see except for clone MT-1 there is no substantial homologous sequences in any of these abortives. ST-1 (our batch of cells) is puzzling; one could argue that it has about $\lesssim 0.5$ a viral equivalents but I don't know whether to believe it. We are repeating it with DNA isolated from the ST-1 you just sent us (and which you said is T-ag negative although morphologically transformed). We plan to run through the rest of the abortives and stables we have to get more numbers but then there is the question of interpretation and what next!

If the result is real (and I tend to believe it at this point) and Smith and Martin's result for SV40 abortively infected cells is also correct, then what is it trying to tell us? Is it that the steps and mechanism of transformation by the two viruses in these hosts is different? Conceivably in PY the integration-excision function (ts-a?) is expressed all the time, both in the "free" and "integrated" state so that a DNA molecule is constantly integrating and excising. Stable transformation occurs only when an integrated genome can't be excised (even though excision "enzyme" is present) or when a genome with a defective ts-a function is integrated and there is a lack of helper particles to complement its excision. In short, wild-type particles can't remain integrated (unless aberrent insertion) but DNA's with a defective ts-a function (as for examples Mike Fried's ts-a itself at high temperature) remain integrated. This model is quite consistent with Vogt and Summers' and Bill Folk's

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recent findings on the "inducibility" of ts-a transformed BHK. In one sense BHK may not be any different than mouse cells except for the inability to multiply the virus. That is, PY BHK is like PY-3T3 and ts-a-BHK is like ts-a-3T3 except that 3T3 is permissive for extensive replication.

What about SV40 infection of 3T3? Perhaps in this instance the integration-excision function is inactivated as a result of integration; that is, integration shuts off expression of the gene controlling the excision function. Thus when growth of the cell dilutes out existing enzyme and helper particles, the integrated genome becomes "locked in". Fusion with permissive cells could activate the excision function and permit replication to occur.

It's quite possible that there are clones of abortively transformed BHK that do contain PY DNA but these might be rare and represent only that class in which either the excision function was defective or the mode of integration was abnormal; in either case the transformed phenotype would have to be "repressed".

The only way I can think of to test this model is to look for curing of transformed cells. I used to think it was best to do this by superinfecting PY-BHK with PY (at high moi) and to see if substantial numbers of non-transformed clones are produced (infection should catalyse a new round of excision-integration leading to a new stable state, about 5% transformed cells). We took a try at this but the attempt was crude, being done with only one strain of PY-BHK; we have not done more. But now I think it should be tried with SV3T3 superinfected with SV40 at multiplicities which cause good "abortive transformation" I shall approach Helene Smith about doing that experiment.

What do you think? and what do you suggest we do further with the analysis of the PY-BHK abortives? Is it worthwhile making another batch of abortives to test? That would take time but maybe it should be done to nail the point down. Perhaps one should use mutagenized PY or preps enriched for defective virus. Conceivably one could reduce the excision process and thereby hope to produce abortives that contain viral DNA.

Are you going to be in the States this summer? At the Tumor Virus meetings in Cold Spring Harbor or at a Gorden Conference? Or is the only way we can discuss this or plan something for me to come to London for awhile? I turned down going to the Brighton Cell Biology meeting (thanks for suggesting my name to them as a speaker) because the first two weeks of September was a very bad time for me to be away. If you're not going to be in the U.S. do you believe it would be worthwhile for me to come during the second half of September for long enough to review and discuss the experiment and how to proceed from here. (Unfortunately I don't think I can stay long enough to do any serious experiments.)

Now for other news. You may recall that I wrote you about the R_I restriction enzyme making one specific double-strand scission of SV40 to create a unique length linear molecule. We've used that molecule now to locate the SV40 segments carried by Ad2-SV40 hybrid ND-1, ND-4 and E46$^+$ along the R_I linear.

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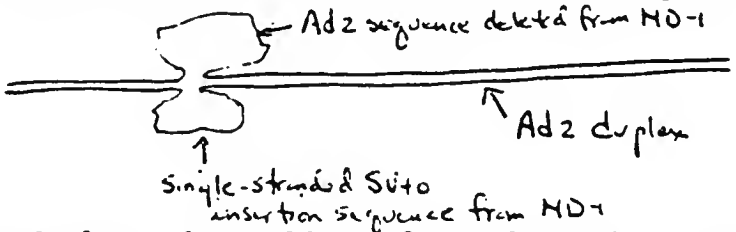
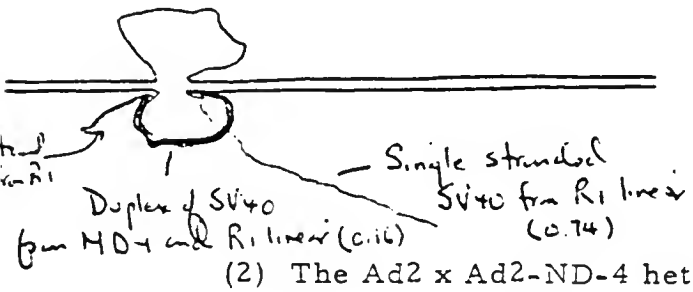
(1) For example the Ad2 x Ad2-ND-1 heteroduplex looks as follows:



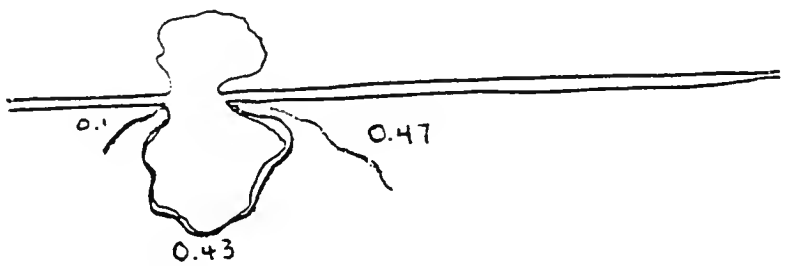
and the heteroduplexes formed by Ad2 x Ad2-ND-1 x R_I linears of SV40 looks like



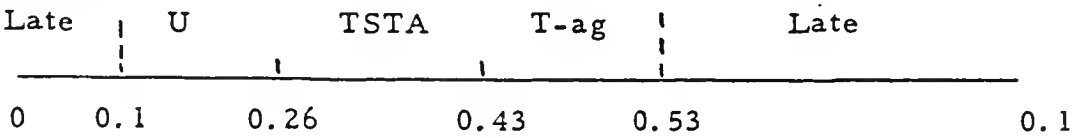
the short arm (0.1) plus the short duplex region (0.16) and the long arm (0.74) equal one SV40 length.

(2) The Ad2 x Ad2-ND-4 heteroduplex is as follows:

and the corresponding Ad2 x Ad2-ND-4 x R_I linear is



According to Lewis and Kelly the AD2-ND-1 induces U-ag while Ad2-ND-4 induces U, TSTA and T-ag. Moreover they say that little or none of the late SV40 genes are carried in these hybrids. Therefore it follows that the R_I restriction enzyme cleaves in one of the "late" genes of SV40 perhaps one of the capsid structural genes. One could draw a tentative "genetic map" of SV40 (not to be taken too seriously) as follows. The actual boundaries of



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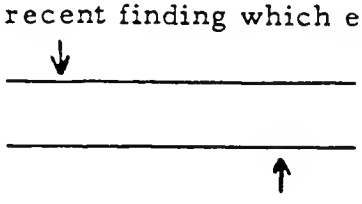
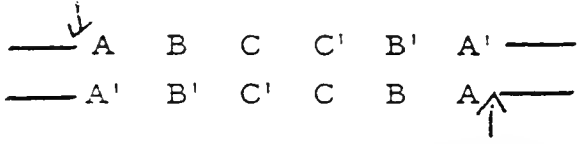
precise boundaries of the

The three putative genes are unclear although the order seems to derive from the fact that ND-1 induces U alone, ND-2 induces U and TSTA but not T and ND-4 induces all three antigens. It is interesting that the segment assigned to late genes is enough to code for about 70,000 daltons worth of polypeptide which would account for the two chains 40,000 and 30,000 that Renato says comprise the capsid.

The heteroduplexes with $E46^+$ are more complex but consistent with this model. The difference is that the SV40 segment integrated in the $E46^+$ hybrid has a deletion of the R_I restriction site but the early genes appear to be contiguous.

The most recent finding which excites us is that R_I makes a staggered break, i.e.,  in which the number of bases between

breaks is of the order of 6 ± 2 (probably six). Thus the linears can be circularized at low concentration at 3-5°C and can be covalently sealed with DNA ligase to regenerate completely infectious and full length SV40 molecules (actually the linears are one-tenth as infectious as wild-type probably because the cell itself can't "close the ring"). It seems very likely that the site at which the enzyme cleaves is identical in all DNA's (it occurs on the average once per 4,000 bases in a random sequence) and very likely is symmetrical.



We can show experimentally, that any two DNA molecules having ends produced by R_I endonuclease can be covalently joined. In other words the ability to construct molecular hybrids is enormously extended. We have now to find out how to deal with this intelligently.

How is the work going on the serum factors? Is it possible to assign specific cellular functions to specific serum proteins yet? You must be just about getting ready to move to the new quarters. Good Luck. I envy Art Pardee his next year.

Well so much for now. I look forward to hearing from you about PY abortives. As we get more data I'll keep you in touch. I'd appreciate it if you could pass some of this scientific information along to Bill Folk and anybody else you care to so as to save me the time of writing it to him.

With best regards to all at ICRF and to Veronica.

Sincerely yours,

Paul

(see next page)

PB:af

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P.S. I wonder if I can make a request of you. So far we've been using one of our PY virus stocks to prepare the P^{32} DNA for annealing. That's not the same one we used to do the infections of BHK. I don't believe that's serious but perhaps we should make some DNA from your stock to be sure. More importantly our virus stock must surely contain defective particles containing insertions of host DNA. This slightly complicates the kinetics of annealing (because the host sequences probably don't anneal with the same kinetics as the PY sequences). If your stock is plaque purified or if you have a plaque purified stock from which you could let us have enough to make a batch of DNA (from infected mouse kidney cells) that would be very helpful. Otherwise we shall have to take the time to make such an isolate and that would be time consuming.

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TABLE I

Cell DNA's tested[1]	Cot #17[2]	Cot #20[3]
	Normalized $Cot_{1/2}$	
Salmon Sperm	2.1×10^{-3}	1.3×10^{-3}
BHK	2.1×10^{-3}	1.3×10^{-3}
SA-2		1.9×10^{-3}
SA-10	2.0×10^{-3}	1.4×10^{-3}
MA-4		1.3×10^{-3}
MA-6		1.3×10^{-3}
MA-8	1.9×10^{-3}	1.4×10^{-3}
ST-1	1.6×10^{-3}	0.9×10^{-3}
MT-1	0.6×10^{-3}	

1. Cell DNA's were added to the annealing mixture at a concentration of $50A_{260}$ (2.5mg/ml); it was sheared to average single-strand chain length of 400-500 bases.

2. In Cot #17 the ^{32}P-PY DNA was at a concentration of $5 \times 10^{-5}A_{260}$ (2.5ng/ml); average chain length of DNA was 400-500 bases.

3. In Cot #20 the ^{32}P-PY DNA was at a concentration of $2.5 \times 10^{-5}A_{260}$ (1.2ng/ml); average chain length same as in Cot #17.

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March 27, 1973

Dr. Norman K. Wessells
Department of Biology
Stanford University

Dear Norm,

I want to nominate Miss Janet Mertz for this year's Francis Lou Kallman Memorial Award. It's my view that Janet is one of the best graduate students presently in our department; in fact, I would rank her amongst the top five students we have ever had in Biochemistry. She is one of the hardest working, brightest and most creative young people I have ever worked with. Moreover, she has that rarest of talents: things get done no matter the difficulties encountered. I predict a very bright and productive scientific career for her future.

Janet came to our Department in September of 1970 with a very distinguished undergraduate record from MIT; she completed a double major in electrical engineering and biological sciences in three years with, as I recall, almost a straight A record. The enthusiasm with which we accepted her has not diminished one iota.

From the day she began research her progress has been most impressive. Her project was to examine the possibility of isolating deletion mutants of SV40 virus and with these to locate the genes codigg for the different viral functions. With almost breathtaking speed she mastered the available literature and set about acquiring the cell culture skills needed to begin her experiments. I was astonished at how rapidly she mastered these techniques and how frequently she came up with improvements in our standard procedures. Now, she is one of the "experts" of our group and I would count heavily on her abilities to train newcomers to the lab.

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Dr. Norman Wessells
Page Two

Janet has virtually completed three publishable and quite significant researches.

1) Together, with Ron Davis, she discovered that the R_1 restriction endonuclease catalyzed cleavage of DNA generates identical and cohesive ends; this makes it possible to reseal the ends of a circular molecule cleaved by this enzyme or, more significantly, to join any two DNA molecules or fragments having such termini (see enclosed reprint). (Yanofsky is presently using this approach for joining the tryptophan repressor gene to a plasmid form of λ DNA so that the number of copies of the repressor gene can be increased in cells made to carry that plasmid.) Her presentation of this work at the Tumor Virus Meeting at Cold Spring Harbor created quite a stir.

2) Together with one of Dale Kaiser's postdoctoral fellows, Doug Berg, she prepared and isolated what has turned out to be an extremely useful new type of genetic structure: λdvgal. In this structure the gal operon of E. coli has been fused into a small DNA plasmid containing about 7% of the λ phage genome. These molecules can be propagated in E. coli cells as episomes (about 50-100 copies per cell). Janet modified and improved an assay system for introducing the pure λdvgal DNA into virgin cells to reestablish the episomal state. This system, therefore, provides a way of introducing new genes into E. coli; by covalently joining any piece of DNA to the λdvgal DNA, it can be introduced and stabilized in the recipient bacterial cell. This ability has paved the way for Dave Hogness' group to attempt to clone Drosophilia DNA segments.

3) Janet is now well along in the main part of her thesis research: The isolation and characterization of deletion mutants of SV40. She has already prepared and characterized stocks with a variety of deletion and substitutions at various locations in the SV40 genome. In doing this she has become an expert and sophisticated electron microscopist and with this technique has carried out an elegant analysis of the heteroduplexes formed from deletion-substitution mutant DNA's with wild-type molecules. Her next immediate goal is to clone these and to characterize the specific genetic defect each type of deletion produces.

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Let me say in finishing that Janet is one of the most eager student teachers we've had. As a first year student she volunteered (she was the only one to do that) to supervise a section of almost 15 students in our Biochemistry 201 course. The following year she participated in literature discussions with another group of Biochemistry students. She generally gives some of the best seminars in courses or in Departmental meetings. Her performance as well as her research has already attracted considerable attention at several National Meetings.

In summary, then, let me say that I know of no student, let alone any woman student, that I feel more confident about in nominating for this recognition. She has certainly earned it.

Sincerely yours,

Paul Berg
Professor and Chairman

PB:af
Enc.

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September 16, 1975

Dr. William J. Rutter
Department of Biochemistry and Biophysics
University of California
San Francisco, California 94143

Dear Bill,

I'm delighted to be able to support your recommendation that Herb Boyer be promoted from Associate Professor to full Professor.

Herb's contributions over the past five years on the mechanisms of restriction and modification of DNA by microbial and phage enzymes have been outstanding. His work combined elegant genetic experiments with enzyme studies to sort out the kind of relationships which characterize the restriction-modification specificities.

About two years ago his work took another and, I believe, more original and germinal line. This was to isolate purified restriction and modification enzymes, to characterize the nature of their cleavages and modifications chemically. During these studies two enzymes (EcoRI and EcoRII) which had been discovered and purified in Boyer's lab were found to have remarkable properties: they cleave both strands of DNA's lacking the appropriate modifications at specific palindromic sequences so as to create cohesive ends. Not only do these enzymes serve as site-specific nucleases, making possible nucleotide sequencing of the fragments but the existence of cohesive ends makes possible in vitro recombination between different DNA molecules having the same enzyme-generated cohesive ends. This may well revolutionize molecular genetics as indicated by the astounding experiment by Boyer and his colleagues in which they succeeded in synthesizing hybrid DNA molecules containing the 16S, 28S or both rDNA of Xenopus ~~covalently inserted into the~~ circular R factor DNA and propagate these molecules in growing E. coli as episomal elements. The implications of this accomplishment are enormous and Herb is actively pursuing many of these new leads.

Boyer's work has had great influence on the newer recognition that restriction enzymes provide us with a very powerful methodology for the analysis of the structure and expression of DNA chromosomes of viruses, bacteria and higher cells. I believe Herb is one of the world's leaders in the field of restriction modification and particularly in its application to other broader problems such as gene expression, chromoson

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structure. His work in the construction and study of recombinant DNA molecules will certainly be at the forefront of this area of biology for a long time to come.

You may recall that I nominated Herb for the 1975 Pfizer Award in Enzyme Chemistry. That is one measure of the high regard I have for his contributions.

I should also add that my scientific contacts with Herb have always been most cordial and helpful. Time and time again he has most generously given us enzyme preparations, bacterial strains and unpublished data. In today's world of "cutthroat" competition, I regard his behavior as being in the best traditions of open science.

With best regards,
Sincerely,

Paul Berg
Professor of Biochemistry

PB:af

Testimony by Paul Berg
Subcommittee on Science, Technology and Space
November 2, 1977

Senator Stevenson, I am grateful for your invitation to participate in this committee's discussion of the current status of recombinant DNA activities. I particularly value the opportunity to present my views on the fundamental and practical issues that have been raised in the public debate on recombinant DNA methods.

To begin, let me introduce myself. My name is Paul Berg and I am Willson Professor of Biochemistry at Stanford University School of Medicine. When I'm not distracted by recombinant DNA matters I conduct research and teach biochemistry and molecular biology. My particular specialties are molecular genetics and viral carcinogenesis, both of which have become increasingly amenable to and dependent upon the use of recombinant DNA methods. I have neither a direct nor indirect association with any commercial enterprise engaged in, or contemplating, research or manufacture using recombinant DNA methods.

I am also not a newcomer to the recombinant DNA controversy. A moment will suffice to summarize the extent of my involvement. My laboratory was amongst the first to construct, outside of a living cell, a hybrid or recombinant DNA molecule; hence, I was one of the earliest practitioners of recombinant DNA research. Because several friends and colleagues expressed concern about the ramifications of my experiments I became an early participant in discussions of their potential risks. Subsequently, my involvement with these concerns grew by being chairman of a committee that warned the National Academy of Sciences about possible risks that might result from the indiscriminate use of recombinant DNA methods. I also served as chairman of the committee that convened and presided over the Asilomar Conference on Recombinant DNA Molecules; the report of those proceedings to the National Institutes of Health made specific and novel recommendations for

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scientific and administrative procedures that could ensure safe conduct of this line of research. Although not one of the architects of the NIH Guidelines, I was consulted at various times during their formulation and prior to their release in July, 1976.

A relevant question with which to begin is why are biologists throughout the world so excited by the recombinant DNA methodology. Is it, as some have charged, just fun and games, the chance to enhance one's career or ambitions or is it to advance genetic manipulation of humans for nefarious purposes? I doubt that any of these selfish reasons motivate more than a small fraction of the international scientific community. Rather, the overwhelming body of scientists view the recombinant DNA methodology as an extraordinary opportunity to solve important biological problems; the knowledge gained will illuminate our biologic nature and heritage;and very likely, help to alleviate the tragedies of human disease, starvation and the pollution of our environment. What are the opportunities, and important biological problems that recombinant DNA research can help to solve? Basically there are three answers:

1) The recombinant DNA methodology permits the isolation of single or groups of genes in high purity and virtually unlimited quantities from almost any living organism. Except in special cases this can not be accomplished by any other presently available method. Coupled with another new procedure, that is virtually child's play, the basic chemical structure of these isolated genes can be readily solved. These two techniques can tell us a great deal about the molecular structure and organization of the complex chromosomes of higher plants, animals and man. I described how recombinant DNA methods were uniquely suited for the task of reconstructing complex chromosomes during my presentation to the National Academy of Sciences Forum on Recombinant DNA Research last March. These are not idle speculations. They are realistic estimates drawn from the impressive achievements so far. There have also been problems and several surprises; each of the surprises introduces unexpected subtleties and makes more fascinating and urgent that we get on with their solution.

2) The ability to join together different DNA molecules per-

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in simple as well as complex chromosomes. Together with classical methods for creating hybrid cells and organisms, one can envision more sophisticated analyses of the mechanism of gene and chromosome function. Understanding differentiation, the process whereby embryonic cells containing the identical complement of genes and chromosomes, gives rise to the myriad cells and organs of the organism, is a worthwhile and realizable goal. It is difficult for me to see how that knowledge will not have ramifications for the treatment and possibly prevention of certain birth defects and other developmental disorders.

3) The ability to isolate pure genes puts us at the threshold of new forms of medicine, industry and agriculture. Tailor-made organisms produced by recombinant DNA methods could provide valuable diagnostic reagents, probes for studying the operational status and efficiency of gene expression in health and disease, vaccines to immunize individuals and animals against the ravages of certain bacterial and viral infections and, possibly, even cancer; and, finally, there is extraordinary progress towards the construction of organisms that make therapeutically useful protein hormones; the isolation of the insulin gene is a promising start; the bacterial production of somatostatin, a hormone produced by the brain is even more astonishing. A joint effort between research groups at the University of California Medical Center, San Francisco, the City of Hope in Los Angeles and the Salk Institute in San Diego has resulted in the production of about 5 mg of somatostatin; only 100 gms of E.coli, grown in about 2 gallons of culture was needed. Bear in mind that it took nearly half a million sheep brains to yield 5 mg of somatostatin in the researches for which Drs. Guillemin and Schalley received this year's Nobel Prize in Medicine. Equally significant is the ingenious and elegant way in which it was accomplished: chemical synthesis of the gene and production of a modified form of the hormone so that chemical processing outside the organism is necessary to liberate the hormone. This approach provides a novel alternative to the previously planned procedures for producing many such products.

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In this brief statement I can only mention but not amplify, some of the important advances that are being made by recombinant DNA techniques. If you like I could expand on some of them in the subsequent discussion. In short, I sense a mounting wave of accomplishment and progess that give lie to the charge that the benefits of recombinant DNA research are only speculative and ephemeral, and that only the dangers are real.

Before considering the question of risks I want to say a few words about genetic engineering, - the directed modification or even construction of new genetic constitutions for animals, plants and man. Partly because of the exaggerated and misleading claims by the popular press and some scientists and laymen as well, this term has evoked as much alarm as excitement. I would guess, that deep down it is what troubles some people most. But man has been involved actively in genetic engineering ever since he came down from the trees, planted maize and domesticated animals. The animals and plants that provide our food, the microorganisms that make our bread, beer and wine, the organisms that make our antibiotics and purify our sewage, are all subject to our genetic counseling. We have carried out wars of genocide against polio virus, small pox and plague and are much the better for it. Recall that for the worst holocaust in history Hitler did not need science and technology; ovens and gas chambers did the job. Malnutrition, poor and inadequate nutrition warps the minds and bodies of hundreds of millions of infants and children throughout the world and our personalities and behavior are manipulated and profoundly influenced by the printed page and television. Genetic manipulation, then, is not, itself, good or bad; we need to distinguish between the acquisition of knowledge and the applications of that knowledge and know how to achieve both wisely. Human genetic engineering is a concept worth examining in rational ways. It is not at all clear that it is feasible, nor when it will be, if at all. There are many difficult and contentious scientific, ethical and moral questions to be examined and at many stages there will be opportunities by all segments of our society to have their say. But preventing or slowing down

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basic genetic research now seems ill-suited to dealing with that question.

Now let me turn to the matter of risks. Three years ago I expressed concern about the use of recombinant DNA techniques. There was no evidence that such experiments were hazardous, only conjecture; but we wanted assurance that these novel experiments would be safe. More than three years later, after considerable discussion by experts in this country and abroad and the analyses of past experiences and new findings, I and others have changed our assessment of the risks. I now believe that the possibility that experimental organisms will be hazardous or released is exceedingly small.

Where it has been examined, organisms modified by recombinant DNA methods are at a disadvantage in competing with their parental or wild organisms. Moreover, certain constructed DNA molecules, hitherto believed to be novel, can arise in nature by reactions akin to those used in the laboratory. There is alsc the virtually unanimous agreement of experts in infectious disease and epidemiology that strain K12, the enfeebled laboratory variant of E. coli widely used for recombinant DNA experiments, is unable to colonize normal human or animal intestinal tracts. Based on recent experiments and existing data, these experts also concluded that there is little or no likelihood that strain K12 can be transformed into an infectious or pathogenic organism or even into a human intestinal inhabitant by a bit of foreign DNA. This view has been echoed by Rene Dubos one of our most eminent biologists, an authority in infectious diseases and an ardent environmentalist. He concluded that "I doubt that gene recombination in the laboratory will create microbes more virulent than those endlessley created by natural processes". Moreover, the introduction of genetically enfeebled derivatives of strain K12 and vectors that are not readily transmissable to other bacteria, provide a further measure of safety. Hence, our initial concern that novel and laboratory-created recombinant DNA molecules could become widely disseminated to man, animals and the ecosystem is not supported by the available data.

Enacting legislation to govern the content and methods of

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In my view legislation of the type that has so far been proposed would inhibit basic research on important biological and medical problems. The rules, procedures, and penalties are predicated on assumptions that will surely change, thereby making it difficult and cumbersome to adjust to the changing information, ideas and opportunities. I believe that legislation could stultify the creativity and initiative that has characterized the development of the recombinant DNA technique; it could also discourage and disillusion young scientists from entering this field. I believe that the present U.S. NIH guidelines, as well as analogous codes of practice in other countries, afford the security to meet the perceived risks. Many scientists believe the guidelines are too restrictive and that most of the proscriptions cannot be justified by any scientific information we now possess. But in spite of their reservations, scientists and their institutions have accepted the guidelines as an interim solution to the anxieties that remain. The acceptance of that view is a responsible action based on careful weighing of the alternatives and rejects irrational fears as a basis for decision.

As I see it, most of us are seeking the same objective: To reap the benefits, basic knowledge and practical advances from recombinant DNA research with a minimun of risk to our world. Members of the academic research community are now the principal practitioners of recombinant DNA research in this country. Since most of their research is funded by government agencies, it is being done in compliance with the procedures and administrative mechanisms embodied in the NIH Guidelines. The sanctions and consequences are severe and, therefore, a strong deterrent to noncompliance. A question frequently put is - what about recombinant DNA activities that are not under the Guidelines' jurisdiction? But surely there are existing mechanisms that guard the public against known hazards of pathogenic agents. Are there not existing statutes that could deal with these hypothetical risks as they do now with real and documented hazards? If not, we could conside establishing a parallel set of procedures and practices, agreed to by representatives of the private sector and monitored by the Department of Commerce, to guide industrial research, development

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and production activities using recombinant DNA methods? Industry's concerns in this area are unique to them; and the academic research community's concerns are foreign to the world of commerce. Does it make sense, then, to have both types of activity operate by an identical set of rules and procedures and subjected to constraints that are inappropriate to each? I suspect that just as the consortium of scientists, the public and the Department of HEW arrived at acceptable codes of practice, a similar coalition of the industrial sector, the public and the Department of Commerce could develop an equally acceptable set of guidelines for their activities.

Let me end by saying that I am particularly concerned by the growing efforts and influence of the anti-science forces.. This is apparent in the increasing pressures to suppress scientists' explorations for fear of what their discoveries will uncover or produce. Decisions and agreements about what is desirable, acceptable and safe to know are nearly impossible to obtain at each level of social organization. Deeply held and conflicting sociopolitical ideals challenge the traditional views of what science is for and how it should be done. As these forces gain momentum, there are increasing attempts to restrict scientific research.

Society desperately requires effective mechanisms for anticipating and evaluating the impact of scientific and technologic breakthroughs. In the recombinant DNA matter scientists demonstrated that they could provide the early warning system for alerting society to the potential benefits and risks of their discoveries; accusations of self-interest, arrogance or even malevolence do little to encourage further efforts of that kind. We may already have squelched the concerned scientist of tomorrow. Governing bodies, everywhere, must seek better ways to encourage scientists' participation and the means to channel their input into the determination of policy.

Perhaps, these poetic words of Aristotle can guide us, scientists and politicians, in our search for wisdom in these matters.

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He wrote:

> "The search for truth is in one way hard and in another easy. For it is evident that no one can master it fully nor miss it wholly. But each adds a little to our knowledge, and from all the facts assembled there arises a certain grandeur."

Thank you.

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STANFORD UNIVERSITY MEDICAL CENTER
STANFORD, CALIFORNIA 94305

TMENT OF BIOCHEMISTRY

PAUL BERG
Willson Professor of Biochemistry

March 5, 1979

Dr. Richard A. Rifkind
Cancer Center/Institute of Cancer Research
College of Physicians & Surgeons of
Columbia University
701 West 168th Street
New York, New York 94305

Dear Dr. Rifkind,

I really wish I had the talent to pick up a dictaphone and provide a scholarly, comprehensive summation of the state of and opportunities in molecular genetic research; but I don't. I also wish I had the leisure to organize various thoughts and opinions on these subjects in a letter, but, alas, that too is lacking. In fact other pressing obligations that can not be put off have precedence on my time and energy, thereby necessitating a brief reply.

In my view the most promising opportunities in molecular genetics research for now and the coming decade will be in the area of organization, replication, expression and regulation of mammalian (human) and other eukaryote genomes. The emergence of restriction endonuclease and molecular cloning techniques, rapid DNA sequencing and the ability to prepare monoclonal antibodies make feasible experimental approaches to many problems, that were hitherto impossible. I believe it will be possible to reconstruct, in molecular detail, the gene organization of specific loci (e.g. the loci governing the expression of the human hemoglobins, the immunoglobulins and HL-A regions are in the offing) as well as extended chromosomal regions. I also believe we may be able to identify the genetic signals and mechanisms that govern differential gene expression (e.g. hormone control, and other homeostatic mechanisms) and possibly to define the general features of developmental programs. Clearly if that comes to pass the impact on our understanding of the underlying mechanisms of many pathologies will be profound. The progress I foresee will enable us to reap the rewards of the basic molecular biology advances of the last three decades.

Without being exhaustive I would identify the following individuals as people capable of making giant strides in that direction: David Hogness, Donald Brown, Richard Axel, Philip Leder, Tom Maniatis, Phillip Sharp, Charles Weissmann, Pierre Chambon, Richard Flavel, David Botstein, Ronald Davis, Gerald Fink. Undoutedly there are others but these will give you an idea of the type of people I have in mind.

Good Luck in your venture,

Sincerely,

Paul Berg

The 1980 Nobel Prize in Chemistry

Three molecular biologists win the prize for discoveries that can be used to study gene structure and control

The current Nobel Prize in Chemistry spotlights contributions to the methodological revolution that is allowing researchers to examine the structure and control of genes of higher organisms in a detail previously unimagined. Half of the prize was awarded to Paul Berg of Stanford University; the other half was awarded jointly to Frederick Sanger of Cambridge University and Walter Gilbert of Harvard. This is Sanger's second Nobel Prize.

Berg is cited for "his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant DNA." According to a press release from the Swedish Royal Academy, "Berg was the first investigator to construct a recombinant DNA molecule, i.e., a molecule containing parts of DNA from different species. His pioneering experiment has resulted in the development of a new technology, often called genetic engineering." Berg does not know whether the Nobel committee had a particular experiment in mind but, he says, "I would like to think it [the prize] was for a body of work and not for a single experiment." Arthur Kornberg, also of Stanford, thinks the only way to interpret the Nobel committee's "carefully worded citation" is as recognition for Berg's 20 years of leadership in the molecular biology of nucleic acids.

In the 1960's, Berg did a great deal of innovative work on bacterial protein synthesis, particularly the interaction of amino acids with transfer RNA's. His work helped explain how these RNA's are used as adapters in decoding. His group and several others also discovered one of the enzymes that copies DNA into RNA.

Then, about 10 years ago, Berg and many other molecular biologists became interested in applying what is known about bacterial gene expression to the study of gene expression in higher organisms. "We began to think of using SV40 [an animal tumor virus] to carry genes into mammalian cells," Berg says. The foreign genes could then be studied and manipulated to see what controls their expression.

In 1971, Berg and his colleagues David Jackson and Robert Symons opened the circular SV40 molecule with a restriction enzyme, Eco R1. This enzyme, which was discovered in Herbert Boyer's laboratory at the University of California at San Francisco, cleaves DNA at specific base sequences. In the case of SV40 DNA, it cleaves it in exactly one spot. Berg's group then spliced the linear SV40 DNA to the DNA of the bacterial virus λ. The λ DNA also is circular and Berg's group cleaved it too with Eco R1.

Although this was the first time that DNA's from two different species were joined, it was not the first time that any DNA's were joined. H. Gobind Khorana, of the Massachusetts Institute of Technology, discovered in the 1960's that an enzyme produced by the bacterial virus T4 can catalyze the linking together of DNA molecules. Berg, Jackson, and Symons enzymatically constructed complementary or "sticky" ends on the two DNA segments to be joined and then used the T4 enzyme to do the joining. The method they used was developed and tested independently by Berg's group and by Peter Lobban and Dale Kaiser of Stanford. Although no one knew it at the time, it was unnecessary to construct sticky ends, since they are automatically produced when Eco R1 cleaves DNA. This fact was discovered in 1972 by Janet Mertz and Ronald Davis and independently by Vittorio Sgaramella, all of Stanford University.

It had been Berg's intention to introduce the SV40-λ hybrid molecule into the bacterium *Escherichia coli*, which λ can infect. In that way, he could get many copies of the molecule to be used for future experiments in gene expression in

Paul Berg

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Wide World Photo

Frederick Sanger

mammalian cells. In the summer of 1971, Mertz, who was Berg's graduate student, described the plan at a tumor virus conference held at Cold Spring Harbor, New York. Robert Pollack of Cold Spring Harbor Laboratories reacted immediately with dismay, pointing out that SV40 transforms human cells in culture and that *E. coli* lives in the human gut. If any *E. coli* infected with the SV40-λ DNA escaped from the laboratory, they could be dangerous.

Berg was persuaded by this argument and decided not to do the experiment. He led molecular biologists in calling for a moratorium on recombinant DNA research until the risks could be assessed and the safety of the experiments ensured. It was a period, Berg recalls, "of more controversy than science." In 1975, the moratorium was conditionally lifted and the National Institutes of Health developed guidelines for the conduct of recombinant DNA experiments. The guidelines have since been softened as the experiments turned out to be less risky than anticipated.

Ironically, the experiment that Berg originally wanted to do would not have succeeded, and no one at the time would have known why. The SV40-λ hybrid would not have replicated in bacteria because Berg inserted the SV40 genes at a site now known to be essential for λ's replication and thereby interrupted this site.

In fact, the heart of recombinant DNA technology is not just gene splicing but also gene cloning. It is necessary to find ways to get foreign genes into cells, ensure that the genes are expressed, and then select for the cells that are expressing those genes. Cloning techniques were pioneered by Stanley Cohen and Annie Chang of Stanford University and Herbert Boyer and Robert Helling of the University of California at San Francisco, who, in the early 1970's, developed a plasmid, which is a small piece of extra-chromosomal DNA, that could carry foreign genes into bacterial cells. The plasmid contained genes that made the bacteria resistant to the antibiotic tetracycline, so that the cells which took up the plasmid and expressed its genes could easily be selected.

In the past decade, recombinant DNA techniques have become increasingly sophisticated. Berg has played a major role in these developments. Most recently, he and others, particularly Daniel Nathans of Johns Hopkins University Medical School, who won a Nobel Prize for his work in restriction enzymes, extensively studied the structure, organization, and replication of SV40 genes. After constructing deletion mutants of SV40 that have proved extremely useful in studies of SV40 gene functions, Berg went back to his original idea of using SV40 to introduce genes into mammalian cells. He spliced to SV40 an *E. coli* gene that allows cells to use xanthine as a substrate in nucleotide synthesis. Then, in separate experiments, he spliced animal genes for globin, histone, or the enzyme dihydrofolate reductase to this hybrid SV40 molecule. When the SV40 carrying the added bacterial and animal genes was introduced into cultured cells, Berg could pick out the cells that were transformed by SV40 by selecting for cells that grow on xanthine. In this way, Berg was able to show that the added animal genes are expressed in cultured cells.

Dean Hamer and Philip Leder of the National Institute of Child Health and Human Development have also used SV40 as a cloning vector in cultured cells. But, says Nathans, "Clearly the notion that you could construct a vector with animal viruses was Berg's idea."

An important aspect of Berg's work has been his extraordinary ability to develop methodologies. For example, he was the first to use nitrocellulose binding assays to study interactions between proteins and nucleic acids. He also developed the nick translation method, which is used to make isotopically labeled DNA probes and is central to current studies of gene functions. "His style of biochemistry helped set the standards in the nucleic acid field," says Nathans.

The second half of the chemistry prize was also given to developers of methodologies. Sanger and Gilbert were honored for their discoveries of ways to sequence DNA. In the past few years, these techniques have become widely used to determine amino acid sequences of proteins because with these methods it is easier and more accurate to sequence the DNA coding for proteins than to sequence the proteins directly. The techniques are also used to determine the intervening sequences that occur in eukaryotic genes and the sequences that occur in control regions of bacterial DNA. By using these methods, molecular biologists hope to learn which sequences control gene expression in higher organisms and how they do so. "DNA sequences are the basic, underlying structures [of molecular biology]. There is nothing more primitive. Your questions are ultimately posed there," says Gilbert.

Sanger and Gilbert are about as different as two scientists can be, and they came upon their sequencing methods by entirely different paths. Sanger is quiet, modest, self-effacing; Gilbert is much more flamboyant. Ted Friedman of the University of California at San Diego, who spent a sabbatical year with Sanger, says, "If you talk to Sanger and do not know who he is, you would think he is the lab caretaker. If you allow him to, he will melt into the woodwork." George Brownlee of Oxford University, who until recently was at Cambridge with Sanger, adds, "Sanger certainly doesn't give himself airs. But in my view, he ranks among the great scientists of our time."

According to Friedman, Sanger's outstanding feature is his "uncanny belief and knowledge that sequencing can be determined by very simple methods." In the 1950's, Sanger studied protein sequencing at a time when no one knew whether all proteins of a particular type

Walter Gilbert

have the same sequences. His first Nobel Prize was awarded for this work. Then he attacked the problem of RNA sequencing, developing the widely used fingerprinting method. About 10 years ago, he set out to sequence DNA, even though this problem, too, was considered intractable.

Sanger's method evolved gradually from more than one line of attack on the problem. In the early 1970's, he discovered the plus-minus sequencing method, a direct precursor of the method he uses today. In the plus-minus method the object is to obtain a set of nested segments of the DNA to be sequenced. The first segment consists of the first nucleotide, the second consists of the first two nucleotides, the third of the first three nucleotides, and so on. These segments are constructed in such a way that the identity of the last nucleotide of each nested segment is known. Once obtained, the nested segments can be separated according to size by electrophoresis on an ultrathin polyacrylamide gel. The separated fragments can be detected because each is isotopically labeled.

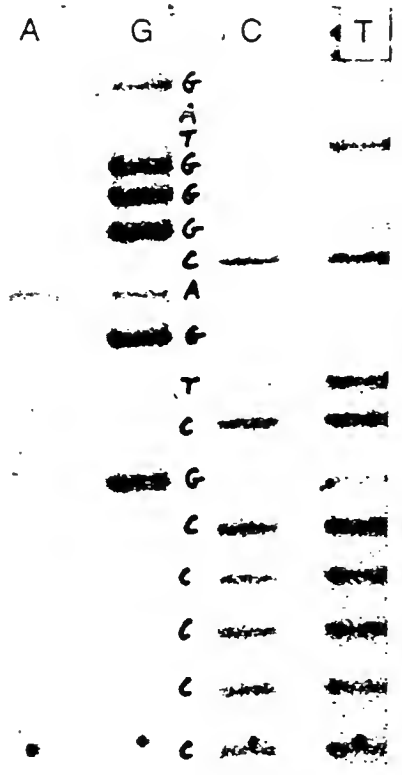
The key to the plus-minus method is obtaining the nested segments. Sanger constructs them by synthesizing them. He separates the two strands of the DNA to be sequenced and then makes partial copies of one of those strands. To ensure that the partial copies include all nested segments and that the terminal nucleotide of each segment is known, Sanger makes the copies under conditions in which one nucleotide is limited in quantity. For example, he provides limited quantities of adenine so that the DNA copying will eventually stop because of lack of adenine. Then all of the copies made will end just before an adenine, and the next nucleotide of each of the resulting segments is adenine. In a similar way, Sanger synthesizes segments ending before each of the other three DNA nucleotides.

Sanger has since improved the plus-minus method to make it more efficient. Instead of supplying limited quantities of each nucleotide, he supplies derivatives of the nucleotides that cause DNA synthesis to stop.

In contrast to Sanger, Gilbert did not deliberately set out to sequence DNA. A highly visible, active scientist who runs a large laboratory, Gilbert has worked on a wide variety of problems in the past 20 years, ranging from how bacterial genes are organized and expressed to gene control in higher organisms and genetic engineering. He is also chairman of the board and cochairman of the board of directors of the gene splicing firm Biogen.

Gilbert, working with Allan Maxam, who is now at Harvard Medical School's Sidney Farber Cancer Institute, came upon a DNA sequencing technique almost by chance. Gilbert recalls that one day in early 1975, Andrei Mirzabekov, of the USSR Academy of Sciences, appeared in his office and urged him to try a new approach to studying how proteins recognize specific sequences of DNA. Gilbert had long been interested in the lac repressor protein of *E. coli*, which binds to the lac operon segment of DNA, and, in fact, it was Gilbert who isolated the lac repressor and operator. Mirzabekov and his colleagues had been probing protein-DNA interactions with dimethyl sulfate, a reagent that methylates the DNA nucleotides adenine and guanine. After reacting with dimethyl sulfate, DNA breaks easily at these bases.

Gilbert decided to expose lac operon DNA to dimethyl sulfate and then break the DNA at adenines and guanines. For comparison, he would bind lac repressor to the operon and repeat the experiment. The adenines and guanines that reacted with the repressor should be protected from the dimethyl sulfate, and so the DNA should not break there. Since the sequence of lac operon DNA was known (it had been copied into RNA and the RNA sequenced), it would be possible to learn where the repressor binds on this DNA.



Part of the sequencing pattern obtained from a piece of DNA about 130 base pairs in length. The letters at the top of the columns, A, G, C, and T (adenine, guanine, cytosine, and thymine) indicate which base was preferentially cleaved by chemicals. The darkest band in each column represents the base missing from the end of the initial segments, with the exception of cytosine (C). All dark bands in the C column represent cytosines even if bands also appear in the T column at that position. Bands that appear in the T column but not in the C column represent thymines (T). To read the sequence of the DNA, read off the base represented by each band, starting from the bottom of the columns. [*Source: Walter Gilbert and Allan Maxam*]

After these experiments, Gilbert and several of his associates discovered a second lac operon of unknown sequence. Maxam repeated the dimethyl sulfate experiments with this new lac operon and the lac repressor. When he and Gilbert saw the results, they realized that they had the beginning of a DNA sequencing method. By using dimethyl sulfate and adjusting the reaction conditions, they could break DNA at either adenines or guanines. Now if they could find a way to break DNA preferentially at thymines or cytosines, they could generate nested segments whose terminal nucleotides were known. With this idea, Maxam set to work to develop ways of breaking DNA at thymines or cytosines. He recalled that under appropriate chemical conditions, hydrazine preferentially weakens DNA at one or the other of these nucleotides. After a summer of work, Maxam succeeded in perfecting the chemical method of sequencing DNA.

The difference between the Sanger method and the Maxam-Gilbert method is that Sanger generates nested segments by synthesizing them and Maxam and Gilbert generate the segments by breaking the DNA at specific bases. Both methods are currently used, and researchers experienced with both say that the choice between them depends in part on the length of DNA to to be sequenced and in part on the personal preferences of the investigator. Tom Maniatis of the California Institute of Technology, for example, uses Sanger's method for very long sequences of DNA because it is faster. For shorter sequences, one or a few genes long, the two methods are comparable in speed, but Maniatis prefers the Maxam-Gilbert method because "Allan has established the protocol so completely that anyone who tries the method is successful."

The full ramifications of recombinant DNA technology and DNA sequencing methods are not yet known. But these techniques are changing molecular biologists' perceptions of what can be learned about the genes of higher organisms.

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