Paul Berg

Moments of Discovery

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Abstract

Devoted teachers and mentors during early childhood and adolescence nurtured my ambition to become a scientist, but it was not until I actually began doing experiments in college and graduate school that I was confident about that choice and of making it a reality. During my postdoctoral experiences and thereafter, I made several significant advances, most notably the discovery of the then novel acyl- and aminoacyl adenylates: the former as intermediates in fatty acyl coenzyme A (CoA) formation and the latter as precursors to aminoacyl tRNAs. In the early 1970s, my research changed from a focus on transcription and translation in *Escherichia coli* to the molecular genetics of mammalian cells. To that end, my laboratory developed a method for creating recombinant DNAs that led us and others, over the next two decades, to create increasingly sophisticated ways for introducing "foreign" DNAs into cultured mammalian cells and to target modifications of specific chromosomal loci. Circumstances surrounding that work drew me into the public policy debates regarding recombinant DNA practices. As an outgrowth of my commitment to teaching, I coauthored several textbooks on molecular genetics and a biography of George Beadle. The colleagues, students, and wealth of associates with whom I interacted have made being a scientist far richer than I can have imagined.

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PROLOGUE

Some years ago, Peter Medawar, a distinguished scientist and philosopher of science, noted that "what scientists do has never been the subject of scientific . . . enquiry." "It is no use looking to scientific papers," he said, "for they not merely conceal, but actively misrepresent the reasoning that goes into the work they describe." What is missing, Medawar observed, is the "unending dialogue between the possible and the actual, between what might be and what is in fact the case." On a related point, Carl Cori, a distinguished biochemist, reflected that "the incredibly rapid advance in many areas of biochemistry and molecular biology makes for equally rapid obsolescence of previous findings. Even the basic observation on which a new advance is based is rapidly forgotten because it has become common knowledge. What may be irretrievably lost," he said, "is the passion, the art, the very flavor that characterizes a particular scientific period; that quickly sinks into oblivion together with the men and women who were the participants."

Aside from trying to forestall the inevitability that my own scientific adventures will sink into oblivion, this essay is intended to supplement the published record with what Medawar refers to as "the unrecorded thoughts and actions that provide the intellectual and experimental underpinnings of scientific investigations." In doing so, I have chosen to focus on a limited number of studies, which I believe influenced the field in which I was working. Regrettably, choosing that course omits the opportunity to identify scientists with whom I carried out several investigations that were important at the time.

As is often the case, only at the onset of my career was it possible for me to conceive and execute experiments on my own. In time, of course, there was a succession of students, postdocs, and visitors whose genius, imagination, perseverance, and stimulation made much of our work flourish. I was also blessed with an amazing group of colleagues (Arthur Kornberg, I. Robert Lehman, David S. Hogness, A. Dale Kaiser, R.L. Baldwin, Melvin Cohen, George R. Stark, and Lubert Stryer), initially at Washington University and later at Stanford University. They created as stimulating, liberating, and congenial an environment for a researcher as one could hope for. Their own accomplishments were both seminal and inspirational.

FAMILY BACKGROUND AND EARLY INFLUENCES

It never occurred to me while growing up to think of my parents as pioneers, certainly

not in the same light as those who crossed our country in covered wagons seeking economic opportunity in the wild beyond. As newly married teenagers (circa 1919) my father Harry, aged 19, and my mother Sarah, a year younger, left their small Russian village on the outskirts of Minsk, knowing that they might never see any of their families ever again. Their destination was New York, but lacking sufficient funds to go there directly, they worked their way across Europe for three years before reaching their embarkation port of Antwerp, Belgium. At some point in their passage through the immigration process, an official changed the family name from Bergzalts (spelled phonetically) to Berg, presumably because that name was common and easier for Americans to spell or pronounce.

I was born June 30, 1926, several years after they settled in Brownsville, a section in Brooklyn that had become the haven for tens of thousands of Eastern European Jews seeking a new life in America. My brother Jack was born 18 months later, and another brother, Irving, was born 5 years afterward. I do not recall much of the period before starting kindergarten—except that going to school forced me to switch from speaking Yiddish, the language my parents used, to English. Public school was not much of a challenge, and I did quite well, at least academically. Report cards that measured monthly performance provided separate grades for academics and behavior; for the first few years, my parents had to contend with As and Ds, respectively, necessitating occasional visits to my teacher to correct my unruly conduct. Nevertheless, the succession of As suggested that I was not challenged sufficiently so I skipped a year with, I suppose, the expectation that it might reduce my troublesome behavior. It worked, and through the fifth grade, my parents took great pride in seeing only As on my report cards.

When I was 10 years old, my family moved to Sea Gate, a gated community at the very far end of Coney Island in Brooklyn. After completing the sixth grade, I attended Mark Twain Jr. High School and was included in a special group that completed the seventh and eighth grades in one year and the ninth, or first year of high school, the next year. My time spent at Mark Twain was an exhilarating period, formative in many ways, largely because of the extraordinary group of students and teachers. It was also the period when I determined that I would become a scientist, although what kind was left to the future. That early commitment was kindled by my innate curiosity and was undoubtedly nurtured by the images and stories of the heroes in Sinclair Lewis's novel *Arrowsmith* and by Paul de Kruif's descriptions of the scientists and discoveries of the microbial causes of disease in *Microbe Hunters*.

Aside from school, sports and girls were most on my mind. Summers were special as the beach was within walking distance from our house and the "summer" girls who arrived for their family holidays competed with the "natives" for our attention. There were the usual seasonal girl friends, one of whom, Beverly Sills, known to all of us as "Bubbles" Silverman, went on to become a distinguished opera diva and promoter. My brother and I played club football on autumn weekends and baseball in the spring. Most of the young people living in Sea Gate who were preparing for college went to Abraham Lincoln High School, at the border of Coney Island and Brighton Beach. Lincoln High drew its students—approximately 4000—from the Coney Island, Brighton Beach, and Sheepshead Bay sections of Brooklyn. Already 8–10 years after it opened, the school claimed many distinguished alumni in the entertainment field. I was pretty good in club football and had great ambitions to make Lincoln's football team. Unfortunately, when I entered as a sophomore, I was just 14 years old and not sufficiently matured physically, either that year or the next, to be competitive with a perennial contender for the city championship.

Instead of football as extracurricular activity, I came under the spell of an inspiring "teacher," Sophie Wolfe. Although her

job was to supervise the stockroom that supplied the classes in chemistry, physics, and biology, she organized an after-school program of science clubs, in part, I presume, because she loved young people. She had a special talent for drawing students out. No question or speculation was spurned; rather, she encouraged us to wonder and to seek problems and solutions for ourselves. Sometimes that meant doing an experiment, sometimes it meant going to the library, but there was always an emphasis on solving problems through investigation. For me, doing experiments to answer questions or solve problems was a very heady experience, almost addicting. Looking back, nurturing curiosity and a passion for seeking solutions were perhaps the most important lessons I gained during that time. With time, many facts that I learned in class were forgotten, but I never lost the urge to question and discover. I was not the only one to benefit from Sophie Wolfe's influence. Two other Nobel Laureates, Arthur Kornberg and Jerome Karle, preceded me through Lincoln High School and Sophie Wolfe's tutelage. Many years later, the New York City Board of Education honored Mrs. Wolfe by bestowing her name on the School's Science Wing; additionally, one floor of the wing was named for Kornberg, one for Karle, and one for me.

A SOJOURN IN THE NAVY

By the time I reached my senior year, the United States had entered World War II. Partly stimulated by the needs of the war effort, I decided to study chemical engineering, and because of my academic record, I was admitted to the City College of New York, one of the nation's most prestigious colleges at the time. But I soon concluded that designing factories for industrial scale chemical processes was far less interesting than exploring the chemical events in biological systems. At the time (circa 1943), the war in the Pacific was not going well, and the Navy was seeking recruits for flight training. Having just turned seventeen and being adventurous and patriotic, I enlisted in the Navy determined to become a fighter pilot. While waiting to be called to service, I withdrew from City College and spent a semester studying biology at Brooklyn College. I then learned through a friend at Pennsylvania State University that their Agriculture College had a major in biochemistry, a subject that I was unaware of, but it seemed close to my interests. I arrived in State College, Pennsylvania, in the autumn of 1943. The Navy called me to active duty after one semester, but I was allowed to remain at Penn State. After a year of preflight training, during which I continued classes in biochemistry, I was reassigned to deck officer school, subsequently commissioned an ensign, and assigned to the antisubmarine warfare school in Key West, Florida. All that training helped me fend off an "attack" by a porpoise one night while I was "officer of the bridge" patrolling in the Caribbean!

With the war's end, my ship, which had been designated to participate in the invasion of Japan, was ordered to Miami for decommissioning. While there and with time on my hands, I visited the city's public library where quite by accident I came across a book titled *The Bacteriophage* by Felix D'Herrelle. D'Herrelle independently rediscovered bacteriophages through their ability to lyse bacteria. I recalled from reading *Arrowsmith* that bacteriophages had been proposed to fight bacterial infections. Eager to learn more about that phenomenon, I read the book from cover to cover and took copious notes for future reference. Many years later, a former postdoctoral fellow, Karl Muench, presented me with that very book when I received the Feodor Lynen Award at a meeting in Miami.

ELECTING A CAREER IN RESEARCH

After being released from the Navy during the summer of 1946, I returned to Penn State and a year later married my longtime sweetheart, Mildred Levy, at the time a newly minted registered nurse. Unable to find work at State

College, she stayed in New York, and I commuted between the two places for all of my senior year. Because graduates in biochemistry at Penn State usually went to work in the food or pharmaceutical industries, I spent summers working at General Foods' and Continental Foods' research labs to obtain experience in these areas. But my goal had long been to conduct independent research, and for that reason, I decided to obtain a Ph.D. degree. While preparing a seminar in my senior year, a group of papers dealing with the use of newly available radioisotopes as tracers for studies of intermediary metabolism caught my attention. Particularly fascinating was a succession of papers from Western Reserve University in Cleveland, Ohio, that showed how compounds labeled with isotopic carbon-14 and nitrogen-15 could be tracked during their conversion from foodstuffs to cellular materials. Although I had never heard of Harland Wood, his leadership in this area of research convinced me that Cleveland should be my next destination.

Only after I arrived in Cleveland did I learn that it was not Wood's Department of Biochemistry that had accepted me. Rather, the Department of Clinical Biochemistry, created especially for Victor Meyers, the former and recently retired professor of biochemistry, was to be my home. Understandably, I was quite disappointed, even more so when Professor Meyers assigned me a thesis problem to measure the cholesterol levels in 85 postmortem hearts to determine if there was any correlation between the amount of cholesterol present and the cause of death. Myers died soon after my arrival, and I was left adrift without a department. Two of Meyers' former students, Jack Leonard and Leonard Skeggs, then assistant professors, invited me to join their efforts to develop an artificial kidney. For the next two years, much of my research involved removing dogs' kidneys and trying to keep them alive by dialysis. On the side, I used the dialysis machine to isolate a variety of physiologically active proteins and peptides from urine. Fortunately, my performance in the biochemistry courses I was taking caught Wood's attention, and he invited me to join the Biochemistry Department and complete my Ph.D. there. This change was a godsend because I could undertake the type of research that had attracted me to Western Reserve.

Until that time, the widely accepted lore was that the methyl groups of methionine and choline could not be synthesized de novo by mammals and had to be supplied in the diet. In vivo experiments had established that methionine formed from homocysteine by transmethylation from compounds, such as choline or betaine, and choline's methyl groups could be derived from methionine. But Warwick Sakami, a professor of biochemistry, and Arnold Welch, a professor of pharmacology, overturned the accepted lore by demonstrating that such methyl groups could be formed from one-carbon compounds, e.g., formic acid and formaldehyde. With Sakami and Professor G. Robert Greenberg as my mentors, I undertook the problem of trying to find out how these C1 compounds are converted to the fully reduced methyl groups in choline and methionine. In the ensuing two years, I showed that guinea pig liver slices could synthesize methionine from homocysteine and ^{14}C-labeled formate (1). My studies established that the three methyl groups of choline are derived by transmethylation from methionine and are not synthesized de novo from C1 precursors (2). Using partially fractionated liver extracts, there were indications that nonprotein factors, possibly derived from folic acid and B_{12}, were needed for methionine synthesis, but I was unable to identify them.

Today, we know a great deal about how formyl tetrahydrofolate, the coenzyme form of folic acid, acts to ferry the various reduced one-carbon units to homocysteine and other acceptors, and how cobalamin, the active form of B_{12}, mediates the methyl transfer. Although formic acid, formaldehyde, and methanol can be converted to methyl groups, the physiologic source of activated C1 units is via the tetrahydrofolate-mediated conversion of serine to glycine. The methylation of a variety

Figure 1

Harland Wood (*left*) and Paul Berg (*right*).

of cellular acceptors, most importantly, proteins and lipids, occurs by transmethylation from S-adenosyl methionine, the product of a reaction of methionine with ATP.

My experience as a graduate student was exhilarating and life altering in that I realized that research in an academic setting was far more enticing and exciting than working in industry. My lab mate, Jerry Hurwitz, shared that passion, and over the years our two families forged a lifelong friendship and enjoyed several foreign travel adventures. But mostly, it was my interactions with Harland Wood that provided the inspiration and set the tone for what was to become my lifelong passion (**Figure 1**). Wood's scientific exploits as a graduate student and postdoctoral fellow at Iowa State University were legendary among the biochemistry graduate students. Wood's devotion to research and to those who shared that commitment showed through his outwardly gruff manner. Hanging out with the graduate students during the many late evenings when he lingered in the laboratory were the experiences I treasured most. His unremitting honesty and forthrightness in the way he practiced science provided the model we all tried to emulate. Although he never received the Nobel Prize for his important discovery of carbon dioxide utilization by organisms other than plants and photosynthetic microbes, he was widely admired and acknowledged as one of the world's leaders in biochemistry. I was told that he published as many or more papers in his eightieth year as the number published that year by the entire biochemistry department.

HERMAN KALCKAR

Herman Kalckar came to the United States in the late 1930s and was forced to remain throughout World War II. During his time in the United States, he was among the earliest to formulate the concept of high-energy bonds as the form in which free energy was captured and stored during oxidative metabolism. He was admired not just for his scientific accomplishments but also for his buoyant and fun-loving manner despite the fact that it was often difficult to understand what he was saying whether it was English or, according to the Danes, when he was speaking Danish. Soon after the war ended, and the situation in Denmark was near normal, Kalckar returned to Copenhagen and established an active laboratory at the university's Institute of Cytophysiology

FROM POSTDOC TO ACADEMIC

Wood determined that I should do my postdoctoral work with Carl Cori, one of the world's preeminent biochemists and a Nobel Laureate. He was distressed to learn, however, that I had already arranged to spend the first of two of my postdoc years with Herman Kalckar (see the Herman Kalckar sidebar) at the Institute of Cytophysiology in Copenhagen, Denmark, and the second with Arthur Kornberg at the National Institutes of Health (NIH) in Bethesda, Maryland. Both men were responsible for notable advances in enzymology, and I wanted to expand my experience in this area of research. Millie and I set out for Europe in the autumn of 1952, and after spending a delightful two weeks with a friend in Oxford and seeing the charms

of Paris, we arrived in Copenhagen. Living in Taarbaek, a small fishing village bordering on the sea and adjacent to the king's private deer park on the outskirts of Copenhagen, was a welcome relief from four years in Cleveland. My 40-minute train ride to and from the institute and the morning and evening bike ride through the woods between our "villa" and the train station provided me with quiet time for preparing and thinking about the experiments of the day. One of our Danish friends speculated that my American Cancer Society fellowship stipend ($3600 per year) might have been more than the King of Denmark's allowance. It certainly allowed us to live well and to sample Europe's sights and culture, which we knew only from reading about them.

The lab was an international mix of postdocs from Canada, Australia, Italy, India, and Scotland with a smattering of Scandinavians (**Figure 2**). At the daily teatime, every person's research progress was discussed, and if there were any exciting results, Kalckar would offer toasts with Aquavit or Cherry Heering. I had the good fortune to pair up with Wolfgang (Bill) Joklik, an Austrian-born Australian who

Figure 2

Members of the Kalckar lab during 1952 and 1953. From bottom left: Vera Hechscher, Agnete Munch-Petersen, Herman Kalckar, Evelyn Smith, Kirsten Lehmann, Beatriz Braganza. From top left: Enrico Cutolo, unidentified, Paul Berg, Eduardo Scarano, Ute Kjeldgaard. Missing from photo are Murray Saffron and Wolfgang (Bill) Joklik.

just completed an Oxford Ph.D. in virology and came to Kalckar's lab to learn some biochemistry. Chasing down one of Kalckar's speculations, Joklik and I discovered a new enzyme that could transfer the terminal phosphate of ATP to any of the ribo- or deoxyribonucleoside diphosphates (3). Although we found the enzyme in yeast and mammalian muscle, it is now known to be ubiquitous in prokaryotes and eukaryotes. Only later did we recognize that this enzyme (we named it nucleoside diphosphokinase or Nudiki for short) was required for producing the ribo- and deoxyribo-triphosphates used for the synthesis of coenzymes, proteins, RNA, and DNA.

While I was still in Copenhagen, Kornberg informed me that he intended to leave the NIH and become the Chairman of the Department of Microbiology at Washington University School of Medicine and invited me to join his group there. Casting aside my reservations about living in St. Louis, I agreed to the change in plans. Later, I learned that I was the only postdoc of four scheduled to come to his lab at the NIH who agreed to the change in venue. We arrived in St. Louis in the autumn of 1953 and found a suitable place to live in University City, a short commute from the Medical Center. The microbiology department was located at the top of an antiquated clinic building, and the shabbiness of the department's setting made it hard to believe this was where Sol Spiegelman and Al Hershey had ushered in molecular biology.

Kornberg's lab was deeply involved in two projects: the path of phospholipid biosynthesis and the synthesis and degradation of pyrimidines. Although he invited me to join his group and to participate in either of those projects, I had already decided on my work. One of the papers that received lots of discussion in Copenhagen was a report from Fritz Lipmann, Feodor Lynen, and their respective collaborators Mary Ellen Jones and Helmut Hilz. That report alleged that the long-standing puzzle of how eukaryotic organisms make acetyl coenzyme A (CoA) had been solved. Lipmann's and Lynen's towering reputations and the novelty of their proposal were enough to capture my attention.

At the time, the synthesis of acetyl CoA in bacteria was known to occur via two separate enzymatic reactions. The first, by acetokinase, involved the phosphorylation of acetate with ATP, and the second, by a transacetylase-catalyzed transfer of the acetyl group from the phosphate to the sulfhydryl group of CoA.

$$\text{ATP} + \text{acetate} \leftrightarrow \text{acetyl phosphate} + \text{ADP}$$
$$\text{Acetyl phosphate} + \text{CoA} \leftrightarrow \text{acetyl CoA} + \text{Pi}.$$

By contrast, in yeast and animal cells, acetyl CoA appeared to be synthesized by a single enzyme, acetyl CoA synthetase, seemingly in a single reaction.

$$\text{ATP} + \text{acetate} + \text{CoA} \leftrightarrow \text{acetyl CoA} + \text{AMP} + \text{PPi}.$$

Lipmann et al. proposed the following steps to account for the overall reaction.

$$\text{Enzyme} + \text{ATP} \leftrightarrow \text{Enzyme-AMP} + \text{PPi}$$
$$\text{Enzyme-AMP} + \text{CoA} \leftrightarrow \text{Enzyme-CoA} + \text{AMP}$$
$$\text{Enzyme-CoA} + \text{acetate} \leftrightarrow \text{Enzyme} + \text{acetyl CoA}.$$

Evidence supporting their proposal relied on the observations that the enzyme alone catalyzed an exchange of ^{32}P-labeled PPi with the pyrophosphoryl moiety of ATP as well as an exchange of ^{14}C-labeled acetate with the acetyl group of acetyl CoA. Both of these findings were consistent with their formulation of three partial reactions and accounted for the energetics and stoichiometry of the overall conversions. Enzyme-bound intermediates were previously suspected as intermediates in certain protease reactions, but none to my knowledge was isolated and characterized. Beyond the novelty of their proposal, I was intrigued by the possibility that analogous enzyme-bound nucleotides might be formed with other nucleoside triphosphates. Could such enzyme-bound nucleotides, I wondered, be activated substrates for nucleic acid synthesis? As a start, it seemed worth trying to isolate the enzyme-AMP compound.

Despite Kornberg's reservations about the validity of the Lipmann-Lynen interpretation, he agreed to my having a go at it with the admonition to purify the enzyme before testing whether their observations and predictions would hold up. Within a relatively short time, using the formation of acetyl CoA as my assay, I obtained a considerably purified enzyme from brewer's yeast. Much to my dismay neither of the two kinds of exchange reactions reported by Lipmann et al. was detectable. Setting about to determine what was needed to reconstitute the ATP-PPi exchange, I confirmed that there was a robust exchange when all the reactants were present but none when acetate and CoA were omitted. Surprisingly, acetate alone was able to completely restore the enzyme's ability to promote the exchange of ATP with PPi. I inferred that ATP reacted with acetate to produce acetyl adenylate, a nucleotidyl analogue of acetyl phosphate, with the concomitant formation of PPi. It was also plausible that as with acetyl phosphate, acetyl adenylate could serve as the acetyl donor to CoA. However, my attempts to isolate the putative acetyl adenylate from the reaction failed.

Seeking to verify my conjecture, I synthesized acetyl adenylate and verified that the enzyme converted it rapidly and quantitatively to ATP with only PPi and to acetyl CoA with added CoA (4). The overall reaction could then be explained as the result of a single enzyme catalyzing two successive steps.

$$\text{ATP} + \text{acetate} \leftrightarrow \text{acetyl adenylate} + \text{PPi}$$
$$\text{acetyl adenylate} + \text{CoA} \leftrightarrow \text{acetyl CoA} + \text{AMP}.$$

A nagging concern was my inability to detect or accumulate acetyl adenylate with only ATP and acetate as the substrates. Perhaps, I thought, it existed only in amounts stoichiometric with the amount of enzyme present. To test that surmise, I resorted to the use of hydroxylamine (H_2NOH), a reagent that was known to react with acetyl phosphate and acetyl CoA to form acetyl hydroxamate and readily confirmed that a similar reaction occurred with synthetic acetyl adenylate. I was elated to detect an accumulation of acetyl hydroxamate and PPi when the enzyme was incubated with ATP, acetate, and hydroxamic acid (5).

$$\text{ATP} + \text{acetate} + \text{hydroxylamine} \rightarrow \text{acetyl hydroxamate} + \text{PPi} + \text{AMP}.$$

Evidently, when CoA, the acetyl acceptor, is absent from the reaction, the acetyl adenylate formed remains tightly bound to the enzyme. Soon thereafter, the formation of acyl adenylates was found to account for the production of the longer-chain fatty acyl CoAs as well as for the activation of other carboxylates, e.g., lipoic acid.

While the work on the acyl adenylates was in progress, Kornberg invited me to join the faculty of the microbiology department. Realizing that I had little experience with microbes, he encouraged me to spend the summer of 1954 with the legendary microbiologist, Cornelius Van Niel, at Stanford University's Hopkins Marine Station in Pacific Grove, California. Spending a summer on the fabled Monterey Peninsula was a memorable and productive interlude before my return to St. Louis, where I was appointed an American Cancer Society Scholar and soon thereafter an assistant professor.

ENCOUNTERING PROTEIN SYNTHESIS

Early in the purification of the acetyl CoA synthetase, I detected a second ATP-PPi exchange reaction; this one specifically promoted by methionine (6). Soon after, other amino acid-dependent exchanges of ATP with PPi were detected in *E. coli* (7). The ability to trap aminoacyl hydroxamates by including hydroxylamine in the reaction mixture was consistent with the likelihood that these enzymes catalyzed the formation of enzyme-bound aminoacyl adenylates. A newly arrived postdoctoral fellow, Fred Bergmann, took on the task of purifying the enzymes involved. Also, reasoning by analogy with the mechanism of

action of acetyl CoA synthetase, it seemed probable that there was a counterpart to CoA, i.e., a naturally occurring acceptor for the aminoacyl moiety, and Jim Ofengand, my first graduate student, set out to search for such an acceptor in *E. coli*.

Bergmann made good progress in purifying several of the aminoacyl adenylate synthetases and with Ofengand discovered that a special fraction of RNA served as the aminoacyl acceptor (8). Purification of the acceptor RNA revealed that it was a relatively small molecule, probably identical to the sRNA that Zamecnik and Hoagland found to stimulate the in vitro incorporation of amino acids into protein. Further work by Ofengand, my research assistant (Marianne Dieckmann), Bergmann, and Jack Preiss (another postdoc) established that a single and highly specific enzyme converts each amino acid to an enzyme-bound aminoacyl adenylate and transfers the aminoacyl group to an RNA molecule, now referred to as tRNA (9–11).

As before, we needed to prove that aminoacyl adenylates were the intermediates in the aminoacylation of tRNA. At Gobind Khorana's invitation, I spent a delightful summer at his British Columbia Research Council laboratory in Vancouver, Canada, where I mastered how to chemically synthesize aminoacyl adenylates, a novel class of molecules (12). With these in hand, each of the purified aminoacyl tRNA synthetases was shown to synthesize ATP in the presence of PPi and formed the respective aminoacyl tRNA with added tRNA (13).

Because the aminoacyl acceptor property of the tRNA could be successively saturated by each amino acid, we inferred that each amino acid is transferred to only a limited set of tRNA molecules. It took other experiments by Preiss et al. (14) to establish that each tRNA molecule had a single acceptor site, somewhat surprisingly one of the two hydroxyl groups of the tRNA's terminal ribose. The stoichiometry of the reaction, one amino acid per tRNA, squared with Francis Crick's surmise that aminoacyl tRNAs serve as "adaptors" that match amino acids to their cognate mRNA codons during ribosome-mediated assembly of polypeptide chains.

Adenylylation of the acyl groups of fatty acids and amino acids by ATP proved to be the first discovered examples of a general class of enzymatic reactions. Proteins, for example, DNA ligase and glutamine synthetase are also adenylylated by ATP. With respect to the ligase action, adenylylation of the protein enables it to catalyze the covalent joining of DNA ends, and adenylylation of glutamine synthetase regulates the responsiveness of the enzyme to feedback regulation. In each case, rapid hydrolysis of the eliminated PPi to Pi drives the adenylylation reactions to completion. In 1959, I received the Eli Lilly Award for the discovery of acyl adenylates.

ARTHUR KORNBERG

During the six years in St. Louis, my relationship with Arthur Kornberg progressed beyond that of student and mentor. He, his wife Sylvy, and their three sons embraced Millie and me as if we were part of their family, and on occasion we traveled and vacationed together. We share a common heritage, a shared philosophy of how to create and sustain the collegiality so essential for a productive research enterprise. We shared the same passion for experimentation, although we often had vigorous debates about whether *E. coli* was the only organism worth studying. I have never known Arthur Kornberg to be unconcerned about the welfare of his students, his colleagues, his friends, and his family. He has been the most influential person in my scientific career, and I suspect that many of the values I hold and actions I took reflected what I absorbed during our association, which until his recent death spanned 55 years.

MOVING TO STANFORD UNIVERSITY

In 1957, Kornberg (see the Arthur Kornberg sidebar) (**Figure 3**) was invited to become chairman of the new Department of Biochemistry at Stanford University's Medical Center in Palo Alto. He accepted the offer and

urged most of the department's faculty at Washington University (Melvin Cohn, David Hogness, A. Dale Kaiser, I.R. Lehman, and myself) to move with him. Shortly before we moved Robert L. (Buzz) Baldwin, a physical chemist at the University of Wisconsin, was invited to join us at Stanford. One justification for moving was the belief that we would be more suited for and more comfortable teaching biochemistry than microbiology, and also the prospect of training biochemistry graduate students was very appealing. In addition, being situated on Stanford's campus opened professional and intellectual opportunities that were lacking at Washington University's Medical Center, which was separated from the main university campus. Many viewed the movement of an entire department faculty from one institution to another as being somewhat unorthodox, which, of course, it was. The move to Stanford in June of 1959 went off without a hitch, and six of the seven who moved remain at Stanford to this day. My wife and I built a house on the university campus and have lived there ever since. Our son John, who was born the year before the move, lives nearby.

The first crop of graduate students that joined the new department was impressive. Michael Chamberlin and William B. Wood, each a recent Harvard graduate in biology or chemistry, chose to join my laboratory group. By 1959, it was becoming clear that the assembly of proteins was dependent on and directed by a new class of RNAs, mRNAs. These mRNAs were surmised to be gene transcripts that, when associated with ribosomes, are translated into proteins. So, Chamberlin set out to purify and characterize the suspected DNA-dependent RNA polymerase that synthesized these mRNAs, and Wood chose to develop a partially purified system from *E. coli* to study how aminoacyl tRNAs are used in the assembly of proteins. In fairly short order, Chamberlin had a highly purified enzyme from *E. coli* that was dependent on a DNA template for RNA formation (15), and Wood prepared a soluble protein-ribosome system capable of synthesizing protein from added amino acids (16).

Figure 3

Arthur Kornberg (*left*) and Paul Berg (*right*).

Sometime in early 1961, possibly in the dead of night, Chamberlin and Wood decided to see if transcription and translation systems could be coupled to achieve a DNA-directed protein synthesis. The outcome was spectacular! By combining purified RNA polymerase and phage T4 DNA with Wood's partially purified protein synthesis system there was a striking increase in the incorporation of amino acids into protein, the increase being entirely dependent on the transcription system and DNA (16, 17). We were, of course excited by this result, and I looked forward to announcing their success in coupling transcription with translation at the 1961 International Congress of Biochemistry in Moscow. But before I could report their results, the Congress participants were electrified by the announcement that Marshall Nirenberg's laboratory found that synthetic RNA polymers, e.g., poly(U), poly(A), and poly(C), could direct the formation of polyphenylalanine, polylysine, and polyproline, respectively. Not surprisingly, Nirenberg's report caused quite a stir because it seemed likely that synthetic RNA polymers would enable the genetic code to be solved quickly. I never did get the chance to report the Chamberlin-Wood findings as

my session was cancelled for the celebration of a returning Russian cosmonaut from space.

Chamberlin went on to characterize the properties of the DNA-dependent RNA polymerase with regard to its activity with different single- and double-stranded DNA templates and to prepare a variety of RNA homo- and copolymers for studies of their biophysical properties (18, 19). Wood continued to characterize the coupled transcription translation system with regard to the DNA and RNA template requirements for promoting protein synthesis (20).

Meanwhile, work on the aminoacyl tRNA synthetase system was accelerating. Ulf Lagerkvist, visiting from the University of Goteborg, Sweden, and Marianne Dieckmann established that the nucleotide sequences adjacent to the invariant 3′-CCA termini of several tRNAs were unique to that particular tRNA's amino acid specificity (21, 22). Over the course of the next eight years, various aminoacyl tRNA synthetases and their tRNAs were purified and characterized (23–29). Although we surmised that aminoacyl adenylates were strongly bound to the synthetase that produced them, it was Anne Norris Baldwin, a postdoc, who demonstrated this using purified isoleucyl tRNA synthetase (30). She obtained the enzyme-bound isoleucyl adenylate complex in pure form and established that ATP was formed quantitatively with added PPi and that isoleucyl tRNA was produced with $tRNA^{ile}$. She also showed that the isoleucyl tRNA synthetase catalyzed the production of enzyme-bound valyl adenylate but failed to transfer the valyl moiety to either $tRNA^{val}$ or $tRNA^{ile}$. Indeed, the addition of $tRNA^{ile}$ induced the hydrolysis of the bound valyl adenylate, thereby revealing a mechanism for avoiding the addition of a structurally related but wrong amino acid onto the wrong tRNA and the consequent errors in synthesized proteins (31).

One of the important unsolved issues regarding the aminoacyl tRNA synthetases was how they recognize those tRNAs specific for their cognate amino acid to the exclusion of all other tRNAs. Using a cellulose filter-binding assay, Michael Yarus, a postdoc, established that the interaction of isoleucyl tRNA synthetase with its cognate tRNAs was profoundly affected by ATP and isoleucine, the enzyme's other substrates (32, 33). Lacking knowledge at that time of the three-dimensional structures of various tRNAs and any means for altering their structure or sequence, we discontinued this approach. Later, we learned that mutational alteration of the tRNA's anticodon sequence impaired its ability to be charged by its cognate enzyme (34).

One of the great pleasures at Stanford has been the very close relationship between the Berg and the Yanofsky families. Charley Yanofsky had joined the Stanford Department of Biological Sciences the year before we had arrived at the Medical Center. We soon discovered our mutual passion for tennis, and during weekends and family holidays, we engaged in some grueling matches whose scores we never recorded. One of the more memorable features of our matches was how much we welcomed the rest periods, some of which lasted longer than our matches. For then, we engaged in discussions about what was going on in our respective labs and the ideas we were exploring. One of these courtside discussions led to one of my most prized collaborations.

Yanofsky was exploring a phenomenon referred to as genetic suppression. Thus, missense mutations in the *E. coli* tryptophan synthetase A protein subunit could be suppressed (overcome) by unlinked mutations that allowed the production of low levels of wild-type protein. One of the missense mutations he was studying (*A36*) resulted from a replacement of glycine at position 211 by arginine. In strains with the suppressor mutation (*su36*), two A proteins were produced, the minor one containing the wild-type glycine and the other with the mutant arginine at position 211. From other data, they surmised that glycine at position 211 in the wild-type A protein was specified by a GGA codon, and the arginine at that position in the mutant protein was most likely encoded by AGA.

Yanofsky's plausible hypothesis was that the suppressor mutation altered a $tRNA^{gly}$ that normally translates GGA as glycine, enabling it to translate AGA as glycine.

John Carbon, a sabbatical visitor to my lab at the time, tested this idea using an RNA copolymer with an alternating sequence of adenine and guanine; this copolymer was synthesized with RNA polymerase and a d(AG:TC) DNA-like polymer as a template, which was provided by Gobind Khorana. Using an in vitro protein-synthesizing system containing wild-type tRNAs, the RNA with alternating AGA and GAG triplets was translated into a polypeptide with alternating arginine and glutamic acid residues. But when the reaction was supplemented with tRNA from an *E. coli* strain containing the *su36* suppressor, gycine replaced some of the arginines in the newly synthesized copolypeptide (35). This made it probable, as Yanofsky surmised, that the suppressor mutation altered a tRNA, most likely a $tRNA^{gly}$, that normally translates the GGA codon as glycine but in its mutated form translates the AGA codon as glycine. Khorana's lab carried out a similar experiment, with a suppressor of a different *trpA* mutant (*A78*), in which glycine at position 241 of the tryptophan synthetase A protein was replaced by cysteine. The presumptive codon for glycine at position 241 in the wild-type protein was GGU and for cysteine in the mutant protein was UGU. In a similar experiment containing wild-type tRNA, the copolymer containing alternating Us and Gs, and, therefore, alternating UGU and GUG codons, directed the formation of a polypeptide with the expected alternating sequence of cysteine and glutamic acid. The same extracts supplemented with tRNA from the strain with the *su78* suppressor incorporated low levels of glycine in place of cysteine into the synthesized polypeptide. Here too, suppression of the *A78* mutation appeared to result from an altered tRNA, in this case allowing translation of the cysteine codon, UGU as glycine.

A later series of investigations by Carbon, Squires, and Charles Hill (summarized in Reference 34) established that the Yanofsky *su36* suppressor mutation altered a $tRNA^{gly}$ that normally translates the glycine codons GGA/G, enabling it to translate AGA; also, the *su78* mutation altered a different $tRNA^{gly}$, the one that translates GGU/C, enabling it to translate the cysteine codon UGU as glycine. These findings raised the question of why suppressor mutations, resulting in loss of the wild-type tRNA alleles, are not lethal. The answer is that they are! These mutants are viable because they also contain the wild-type $tRNA^{gly}$ allelle (36).

Larry Soll, a graduate student, arrived at a similar conclusion in his discovery of the *su7* mutation, which suppresses unnatural protein chain termination by the insertion of glutamine at mutationally introduced UAG codons (37, 38). He found that cells, containing the *su7* mutation, were viable only if the wild-type allele was retained. Moshe Yaniv, a postdoc visitor from the Pasteur Institute, determined that the *su7* mutation altered the anticodon of a $tRNA^{trp}$, enabling it to translate UAG instead of UGG (39). Quite surprisingly, that particular change in the $tRNA^{trp}$ anticodon impairs its ability to react with the tryptophanyl tRNA synthetase but enables it to accept glutamine from the glutaminyl tRNA synthetase. This finding demonstrates that the tRNA's anticodon contributes to the interaction with the appropriate aminoacyl tRNA synthetase. The inability of the mutated $tRNA^{trp}$ to translate UGG, the only known codon for tryptophan, is lethal, and therefore the gene encoding this tRNA can only be maintained in organisms that also contain the wild-type $tRNA^{trp}$ allele (38).

CHANGING RESEARCH FOCUS

With the solution of the genetic code and a fairly convincing outline of the mechanisms of gene expression and regulation in prokaryotes, it seemed reasonable to wonder if the deductions from these studies were applicable to eukaryote organisms, mammals in particular. Because I was well aware of how important the

studies of bacteriophage-bacterial cell interactions had been in developing our knowledge of prokaryotic molecular biology, it seemed reasonable to study the interactions of DNA tumor viruses and cultured mammalian cells. I was drawn to this problem by my colleague, Dale Kaiser, who noted the striking similarity between the phenomenon of bacteriophage-induced lysogeny in prokaryotes and the oncogenic outcome following infections of mammalian cells by polyoma and SV40, two small DNA-containing viruses. Both appeared to result from the integration of viral DNA into the transformed cell's genome and the continued expression of one or more of the virus's genes.

An intriguing feature of both polyoma and SV40 was their small DNA genomes (5–7 kbp); both viruses appeared to contain only five genes organized as a covalently circular double-stranded DNA. Because Renato Dulbecco's lab at the Salk Institute in La Jolla, California, was using mammalian cell cultures to explore the mechanism of polyoma's and SV40's oncogenicity, I decided to spend the 1967–1968 academic year in his lab. Besides arranging for my own stay, Dulbecco graciously permitted me to bring Marianne Dieckmann, my longtime assistant, and Francois Cuzin, a postdoc from the Pasteur Institute, scheduled to join my lab that year.

Dulbecco's lab already established that polyoma could be propagated in and kill mouse cells. By contrast, the virus fails to replicate in hamster or rat cells; instead, at some low frequency, the surviving cells are stably transformed to a tumor-like state. Transformation, it was believed, was a consequence of integration of the viral DNA into the cell's chromosomes and of the continued expression of one of polyoma's proteins—the large T protein. Earlier, Marguerite Vogt, Dulbecco's longtime research associate, had obtained a rare polyoma-transformed mouse cell line by infecting 3T3 mouse cells with a thermosensitive replication-defective polyoma and maintaining the culture at 39°C. The surviving cells remained transformed and virus free when grown at 39°C, but the cells lysed and produced mature infectious virus at 31°C. As this response was unusual, our Stanford group under Vogt's tutelage set out to characterize the nature of the induction process.

We confirmed that, within a few hours after shifting the cells to 31°C, covalently closed viral DNA molecules could be detected. Curiously, in addition to the normal-sized viral DNA (~5200 bp), there were substantial quantities of dimer- and trimer-sized polyoma DNA molecules (40). Considering that oligomeric forms of polyoma DNA were never detected during normal virus DNA replication, we reasoned that the oligomeric forms were a consequence of an early event of the induction process. And, because in many instances viral DNA is integrated into chromosomes in tandem arrays, we reasoned that the various forms of oligomeric DNA arose by replicative or recombinational excision from dimeric, trimeric, or longer tandem repeats of the integrated viral sequence (40, 41). My stay and collaboration with the Dulbecco lab were both inspiring and gratifying, so much so that when I returned to Stanford, I established my own research program on the molecular biology of tumor viruses focusing on SV40.

While at the Salk Institute, I was struck by findings reported from several labs that polyoma or SV40-infected cell cultures contained variable quantities of *pseudovirions*, that is, virus particles containing cellular DNA. Those reports were reminiscent of the findings that infections of *E. coli* with bacteriophage P1 yielded phage particles containing bacterial DNA as well as normal virus DNA. Such pseudophage can transfer bacterial genes to newly infected cells, a process referred to as transduction. Indeed, during my collaboration with Yanofsky, I frequently used P1 phage-mediated transduction to alter the genetic makeup of the strains we worked with. Fully appreciative of how powerful this capability could be, I considered the possibility of trying to use polyoma or SV40 to recover genes from infected mammalian cells

and quite possibly to transfer isolated genes to other cells. But given the complexity of mammalian DNA, the small amount of DNA that can be included in either of polyoma or SV40 particles (5–7 kbp) and the lack of ways to select pseudovirions bearing specific genes, it seemed highly unlikely that any particular gene could be recovered or detected in this way.

An alternative approach was to construct SV40 genomes containing foreign DNA in vitro and to introduce such recombinant DNAs into cells by DNA transfection. Because SV40 DNA is covalently circular, this would involve opening the ring molecule and linking new DNA to its ends. The first issue was what foreign DNA to introduce into SV40? As there were no isolated mammalian genes at that time, we settled on a small circular DNA plasmid that contained λ bacteriophage genes that enable it to propagate in *E. coli*. That plasmid (λ*dvgal*) also contained three *E. coli* genes responsible for galactose utilization. Because λ*dvgal* could replicate in *E. coli*, we presumed that the proposed SV40 recombinant could as well, and so we might be able to determine if SV40 genes could be expressed in a bacterium. Furthermore, we wanted to learn if the bacterial genes could function in a mammalian cell.

The principal experimental question was how to join these two DNA molecules in vitro? We were aware from the work of others, notably that by Dale Kaiser, that the short complimentary single-strand ends of linear phage λ DNA can be joined to make circles and that "end-to-end joining" of phage DNA can produce long linear chains. Thus, it seemed plausible that if we could attach single-strand extensions of only As at the two 3′ ends of one duplex DNA and single-strand extensions of only Ts at the two 3′ termini of another duplex DNA, the two DNAs would associate via complementary base pairing of their respective poly A and poly T "tails." We surmised that any gaps would be filled in with DNA polymerase and that any remaining nicks would be sealed with DNA ligase.

The task of implementing that strategy fell to David Jackson, a postdoc, and Robert Symons, a sabbatical visitor from Adelaide, Australia. The strategy and the details of how the first recombinant DNA, SV40λ*dvgal*, was produced and characterized are described in a paper by Jackson et al. (42). Because the λ*dvgal* plasmid and SV40 DNAs were circular, each had to be converted to linear molecules before they could be modified for joining. John Morrow, a graduate student, discovered that *Eco*RI endonuclease cleaves SV40 DNA once at a unique site to produce linear molecules (43). Douglas Berg, Jackson, and Janet Mertz (a graduate student) characterized the λ*dvgal* plasmid DNA and showed that it too is cleaved once to create a unique linear molecule by *Eco*RI endonuclease. Unaware at the time that cleavages with *Eco*R1 produced complementary single-strand ends (see below), Jackson and Symons enzymatically synthesized short lengths of As on the 3′ ends of SV40 DNA and short lengths of Ts on the 3′ ends of λ*dvgal* DNA. The two DNAs were annealed, any gaps at the dA:dT joins were filled by incubation with DNA polymerase and the four deoxynucleoside triphosphates, and the SV40λ*dvgal* recombinant was covalently closed by the action of DNA ligase (**Figure 4**). Our work along these lines was facilitated by interactions with Peter Lobban, a graduate student in Kaiser's lab, who independently and simultaneously hit on the same idea for covalently joining two DNAs.

There were some who reckoned that the construction of the SV40 recombinant was a reckless act because of its ostensible ability to replicate in *E. coli* and thereby spread a risk of the bacterium escaping into the environment and potentially produce a cancer contagion. The debate was heated but relatively brief as we deferred testing the biological properties of this recombinant. But the same concerns emerged in a different context about a year later when simpler methods were developed for creating and propagating recombinant DNAs (see below).

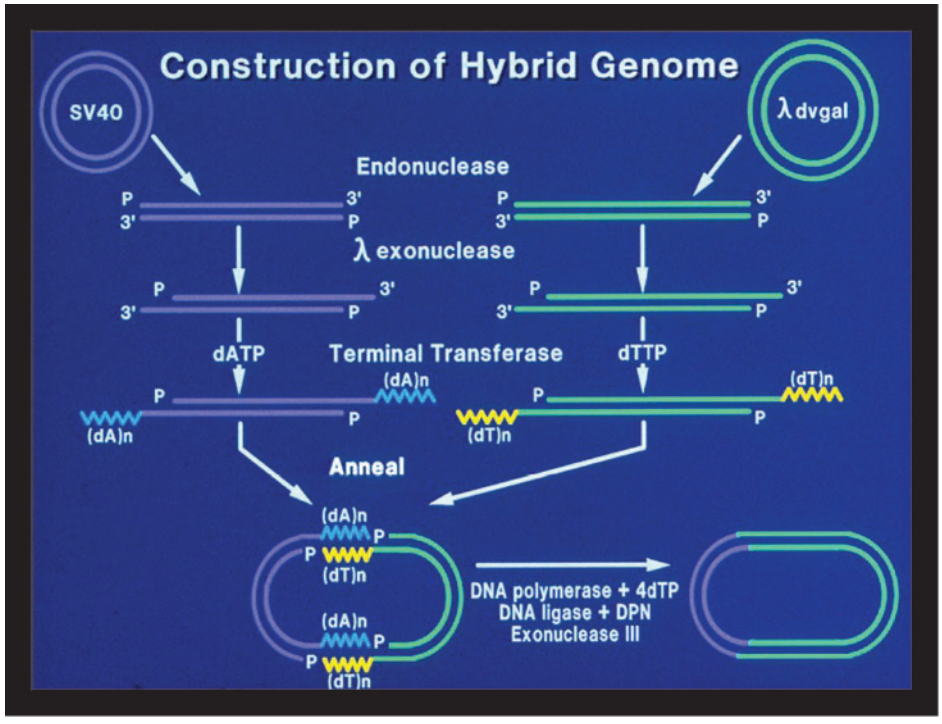

Figure 4

Construction of SV40λ*dvgal*. Conversion of the circular SV40 and λ*dvgal* plasmid to linear DNAs, then modification of the ends by treatment with λ 5′- to 3′-exonuclease followed by addition of short tails of As to the SV40 DNA ends and comparable short tails of Ts to the λ*dvgal* DNA ends. The two modified DNAs were annealed to form hybrid molecules held together by short bridges of dA:dT. Any gaps (because of unequal lengths of As and Ts) were filled, and the ends were ligated as indicated to yield the covalently closed SV40λ*dvgal* hybrid DNA (42).

THE NOBEL EXPERIENCE

It was for my work initiating the development of the recombinant DNA technology that I received the 1980 Nobel Prize in Chemistry along with Walter Gilbert and Fred Sanger, each of whom developed separate rapid and efficient methods for sequencing DNA. In a sense, the Nobel Committee acknowledged that the two developments were complementary because each technological advance would not have been as valuable without the other. The ability to sequence whole genomes of a variety of organisms relied on this dual capability.

From the moment I was notified of the Prize by a telephone call at about 5:00 A.M. from Arthur Kornberg, who learned of it from an early morning news announcement, the sense of unreality and self-consciousness was overwhelming. Those feelings soon dissipated in the frenzy of press interviews, telephone calls, and partying by friends and colleagues. When things quieted down, there was the need to prepare for the trip to Stockholm and for the festivities and numerous functions that make Nobel Week so special. From the moment of our arrival until we departed, my family and I were engaged by receptions, dinners, and trips to Stockholm's tourist attractions. Being properly attired, and in the right place and on time, was overseen by a lovely young woman from the Foreign Service whose responsibility it was to brief us on the time, place, proper dress, and etiquette for the

various social events. I was kept busy by a round of interviews at which the Swedish press asked Sanger, Gilbert, and me to explain the meaning and significance of our respective accomplishments. Additionally, we each gave a lecture at the Academy of Sciences. One of the highlights for the Swedish populace was the televised roundtable at which all of that year's Laureates engaged in an expansive give-and-take discussion of such matters as foreign affairs and futurology.

The formal award ceremony took place in Stockholm's elaborately decorated Royal Concert Hall where, in addition to the family and friends of the Laureates, Sweden's scientific elite and the royal family were gathered. A trumpet's blare signaled the entry and seating of the Laureates. By tradition, winners of the physics prize entered first, followed by the chemists, then by the winners of the physiology or medicine prize, and the literature and economics prize winners in that order (**Figure 5**). The King and Queen, flanked by numerous Swedish and foreign diplomats and representatives from the various Nobel Committees, were seated on their thrones opposite the Laureates. Each Laureate, in turn, was summoned to center stage for a recitation of their accomplishment and the bestowing of the gold medal and certificate from the King. At the ceremony's conclusion, the families and friends of the winners rushed to the stage for the hugs and kisses.

The ceremony was followed by a dinner at Stockholm's grand City Hall. Its blue room's golden glow, enhanced by the candlelight and by a profusion of flowers provided by the citizens of San Marino, Italy, where Nobel died, is not easily forgotten. Orchestral music signaled the time for the King to lead the entourage down the grand stairway where nearly 1500 notables were already seated. Then the Laureates entered, each with a lady of the royal family on his arm, followed by their wives escorted by men of the King's family. Orchestral music and student-led choral groups serenaded the assembled diners throughout the meal. Customarily, the Laureates address the gathering near the end of dinner. I spoke for the chemists, and Sanger, a two-time winner of the Prize, addressed his remarks to the assembled university students.

The next night, the Laureates and their wives dined in the Royal Palace with the King and Queen. The remaining days were filled with shopping and partying. Early one morning, Millie, John, and I were awakened by a parade of beautiful young women, wearing crowns adorned with burning candles, bearing coffee and cakes to our rooms and singing the Santa Lucia hymn that celebrates the beginning of the Winter Festival of Light. Crowning the social events of our week's stay was the traditional formal dinner dance and high jinks with Stockholm University students. No one who has received that high honor is left unchanged; the notoriety and privileges that follow can be both overwhelming and intrusive.

GENOMIC ANALYSIS OF SV40

While the recombinant DNA experiment was under way, progress was being made in identifying SV40's genes and their transcripts. Several laboratories obtained temperature-sensitive mutants (*ts*) that affected both early gene expression and DNA replication; so-called late region *ts* mutants that also affected the production of the virion's three capsid proteins were also identified. A significant advance enabling SV40 genes to be localized on the DNA was facilitated by Kathleen Danna & Daniel Nathans' finding that the viral DNA is cleaved at 11 sites with a mixture of *Hind*ll and *Hind*lll endonucleases. Of greater importance, the resulting fragments could be ordered, allowing for the construction of a physical map of the SV40 genome. Using these separated fragments as hybridization probes, they identified the regions transcribed early and late after infection as well as the origin of bidirectional DNA replication. That work emphasized how valuable a detailed physical map of the viral DNA could be for assigning physiological functions to specific regions of the virus' genome.

Figure 5

Nobel Prize Award Ceremony 1980. The Nobel Prize given to me (*left*) by King Carl XVI Gustaf (*right*).

Because SV40 DNA is circular, a reference site for map coordinates was needed to locate the various endonuclease cleavage sites and, subsequently, specific genetic loci. The single *Eco*RI endonuclease cleavage site served that purpose. Soon thereafter, additional restriction endonucleases, e.g., *Hpa*II, *Bam*H1, *Taq*I, *Bcl*2, were also found to cleave SV40 DNA once, providing additional sites for constructing an overall map. What was needed, however, was a way to define the physical boundaries of the viral genes, more specifically the mRNAs and protein-coding regions. For that we opted to create various size deletions,

insertions, and duplications throughout the genome and determine what function(s) was lost with each. The virtue of this approach over relying on spontaneous conditional mutations is that the effects of the mutations are less likely to be leaky, and the location and extent of the deletions could readily be established by restriction enzyme analysis and electron microscopy of DNA heteroduplexes.

Initially, Mertz, and subsequently others in the lab, introduced deletions at or between already mapped restriction sites (44–47). Subsequently, a large collection of mutants with various size deletions throughout the viral DNA was prepared, using limited cleavage with DNase and relying on transfected cells to seal the ends (48, 49). Most of the deletion mutants were nonviable and could only be isolated by propagating them together with complementary *ts* mutants (45, 46). Thus, deletions affecting an early function were propagated at elevated temperatures with a *ts* late mutant; deletion mutants impaired in a late function were propagated with a *ts* early mutant. Some of the deletions yielded virus isolates that were viable, thereby identifying segments of the viral DNA that were dispensable for growth (49). Mapping the deletion sites of both kinds of mutants provided the first physical/genetic map of the SV40 genome (50).

One intriguing but puzzling finding was that a closely bunched group of deletions was mapped within the region believed to encode the large T protein, the entity known to be essential for replication of the viral DNA and the virus's oncogenicity (49). Nevertheless, these mutants were clearly viable and multiplied as well as the wild-type virus. Moreover, all of these mutants produced the normal-sized large T protein. The answer to this conundrum emerged from the discovery of introns and splicing. Only then did we appreciate that the coding sequence for the large T protein is interrupted by an intron and that this class of deletions fell within that intron (51). This explained why these mutants were viable and produced a full-size large T protein. Another surprise was the realization that the SV40 early region expresses two proteins: the large T protein and a small t protein. As it turned out, the small t protein is encoded by a second alternately spliced mRNA that retains part of the large T intron sequence (51). The small t protein's function is currently believed to contribute to the virus's oncogenicity, although the mechanism for its action remains unclear. Naturally, we were chagrined at having missed discovering the existence of introns and the phenomenon of alternate splicing to produce related but distinctive mRNAs, each encoding related but distinctive proteins.

CREATING NOVEL TRANSDUCING VECTORS

Our ability to propagate nonviable SV40 deletion mutants by complementation with appropriate *ts* mutants suggested that we could also propagate recombinants in which various segments of SV40 DNA were replaced with foreign DNA. In the first such experiments, Steve Goff, a graduate student, replaced almost the entire late region with a segment of λ phage DNA (52, 53). Although the resulting recombinants could be propagated in monkey cells with a *ts* early mutant as a helper, there was no detectable λ phage RNA produced in infected monkey cells. By contrast, Richard Mulligan, a graduate student, constructed a recombinant that contained a rabbit ß-globin cDNA in place of the VP1 coding sequence. That recombinant produced copious amounts of ß-globin mRNA and ß-globin protein following infections (54). Expression of the ß-globin cDNA was very dependent on where the cDNA was inserted in the late region. At some locations, there was no ß-globin RNA or protein made, whereas at other sites, expression was robust (55). The explanation for that variability emerged from the discovery that all SV40 mRNAs transcribed from the late region promoters are products of differential splicing. Thus, the mRNAs that encode either the VP1 or VP2/3 capsid proteins each contain a common 5′ leader segment spliced to the start of the coding sequence for each of

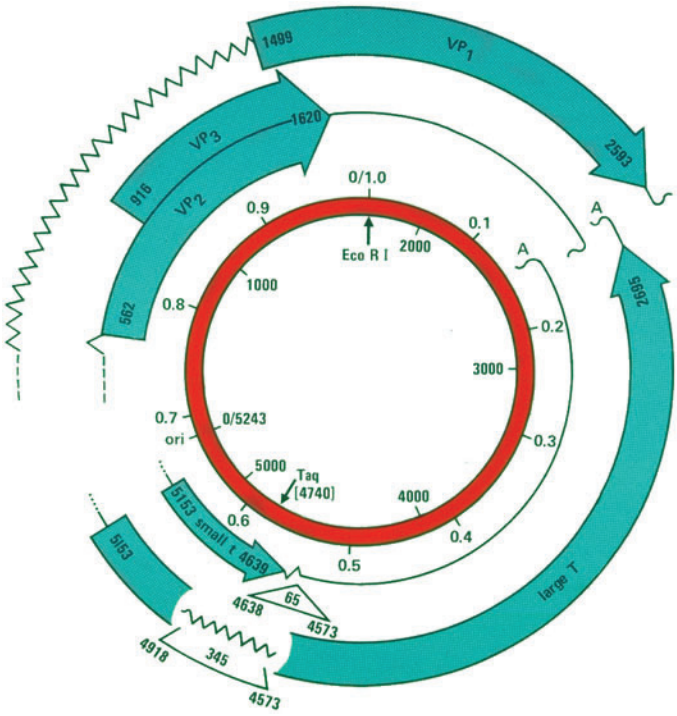

Figure 6

Map of expressed regions of SV40 DNA. The inner circle represents the genomic DNA. The numbers on the outside of the circle are the fractional distances from the single *Eco*RI cleavage site, and the numbers on the inside of the circle indicate the genome's 5243 base pairs, clockwise from the origin of replication, ori. The coding segments for the early proteins (large T and small t) and the late proteins (VP1, VP2, and VP3) are shown as shaded regions. The early region mRNAs are shown counterclockwise, and the late region mRNAs in the clockwise direction originating from their respective promoters adjacent to ori. The 5′ ends of the mRNAs are represented as dashed or dotted lines, the spliced out segments of the early and late mRNAs are represented as wavy lines, the 3′ noncoding segments are shown as straight lines, and the wiggle at their other ends are the poly A ends. Note that the early and late region transcripts are alternately spliced to yield different mRNAs.

the proteins (**Figure 6**). For reasons that are still unclear, splicing of the primary transcript is essential for obtaining stable mRNA, and removal or alteration of the splice junctions prevents the accumulation of mRNA (56). Thus, the SV40 λ DNA recombinant lacked the late region splicing junctions, and they were intact in those ß-globin recombinants that were expressed. Consequently, to express a foreign sequence in the SV40 late region, the insert has to be positioned so that it can be transcribed from the virus' late region promoter and undergo a splicing event. Furthermore, for the inserted sequence to be translated into protein, its start codon must be the first AUG from the 5′ end of the mRNA (54). The same splicing and positioning requirements governed the construction of recombinants that contained the foreign DNA sequence in the early region. Thus, ß-globin could be expressed only if its AUG, and coding sequence, was the first AUG from the mRNA's 5′ end, and the early region intron was included in the transcript (57, 58). Additional experiments carried out by Andrew Buchman, a graduate student, demonstrated that any spliceable intron, placed at different sites within the ß-globin cDNA or even 3′ to the coding sequence, promoted robust accumulation of ß-globin mRNA (59).

Taking account of these constraints, we prepared SV40 recombinants containing a gene encoding mammalian dihydrofolate reductase (*dhfr*) cDNA (60), a bacterial gene encoding the xanthine-guanine phosphoribosyl transferase gene (*gpt*) (61) and the bacterial gene for aminoglycoside phosphotransferase (*neo*) (62). Mammalian cells that acquire the *gpt* gene can be propagated in a medium containing mycophenolic acid (an inhibitor of mammalian hypoxanthine/guanine phosphoribosyl transferase) and xanthine (63). Cells that lack the *gpt* gene fail to survive in that medium because the mammalian cells cannot use xanthine for their purine requirement. Similarly, expression of *neo* in mammalian cells confers resistance to G418, a toxic aminoglycoside for eukaryotic cells (62). Recombinant DNAs containing either *gpt* or *neo* were especially important because they enabled selection for cells that acquired and expressed the bacterial genes irrespective of whether the genes were maintained as a plasmid or incorporated into the chromosomes (see below).

To eliminate the necessity for a complimentary helper virus to propagate recombinant viruses, we prepared recombinant DNAs that could be assembled in vitro, propagated

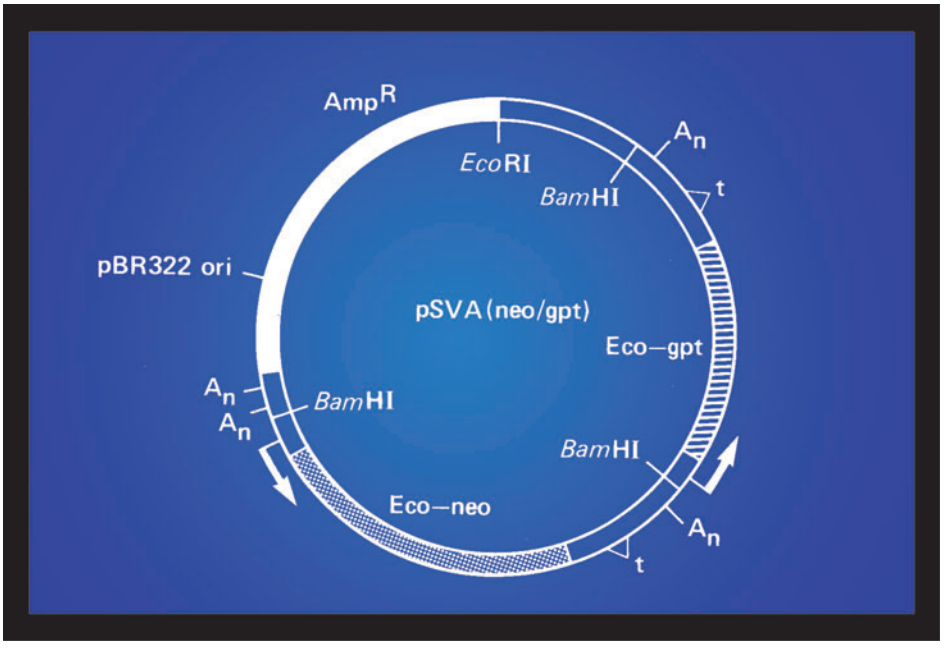

Figure 7

pSV2*(neo/gpt)*. The plasmid's circular DNA is drawn as a circle. The solid segment represents the bacterial plasmid contribution (pBR322) with its origin of replication (ori) in *E. coli*, and the gene conferring resistance to ampicillin (*AMP*^{R}). The cross hatched and slashed regions represent the coding segments of the *E. coli neo* and *gpt* genes, respectively. The remaining DNA is derived from SV40. The position of the arrows and their direction specify the location of the early region promoter and the direction of transcription, respectively. The symbol t and small triangle show the position of the intron that is spliced in the small tmRNA (**Figure 6**). The segment specifying polyadenylation is shown as A_n.

in *E. coli*, and, following their isolation, could be used to transfect mammalian cells. To that end, we replaced a dispensable segment of pBR322 DNA with a DNA segment containing the SV40 early region promoter and origin of replication (ori), an expressible coding sequence, for ß-globin or DHFR but generally *gpt* or *neo*, an intron located 3′- to the coding sequence, and a segment specifying the early region transcript site of polyadenylation (**Figure 7**). The pSV2 recombinant replicates in *E. coli* and cells containing it can be selected using media containing ampicillin. The genes linked to the SV40 early promoter are transcribed in most mammalian cells, and the mRNA is processed and translated. Moreover, because it contains the origin of replication, pSV2 replicates in cells that express the large T protein.

The pSV2 derivatives proved to be extremely useful for a number of investigations. For example, the pSV2 recombinant plasmids expressing ß-globin or DHFR were used to define structure-function relationships of the early promoter and ori (64). By making small and extended deletions in the promoter, Michael Fromm, a graduate student, identified the regions essential for replication and transcription, the most startling of which was the discovery of enhancers. His key finding was that deletion of the repeated 72-bp segment adjacent to the early region promoter prevented transcription of the linked ß-globin sequence. Somewhat unexpectedly, transcription was restored if the 72-bp segment was inverted at its normal location. More surprising was Fromm's finding that the 72-bp segment activated transcription when inserted in the

pBR322 DNA several kilobases distant from the promoter (65). Thus, the 72-bp segment enhanced transcription irrespective of its orientation and distance relative to the promoter. Later, other investigators identified functionally similar enhancer sequences and the proteins (transcription factors) that bind to them. The pSV2 ß-globin and pSV2*gpt*, recombinant plasmids, were also used by Buchman and Fromm (66) to determine how the large T protein regulates early region transcription both before and after the onset of DNA replication. Tom Kadesch, a postdoc, determined how the position of a single enhancer element influences the transcription of tandemly arranged *gpt* and *neo* transcription units (67), and David Peabody and Suresh Subramani, both postdocs, determined the requirements for translation termination and reinitiation of sequential protein-coding sequences (68). Using pSV2 ß-globin plasmids that lacked an intron, Buchman established that the splicing requirement could be satisfied by insertion of either of the two ß-globin gene introns into the cDNA; unsurprisingly, preventing splicing by mutating the splice junctions of any one of the introns markedly diminished mRNA production. Splicing occurred normally even if introns from other genes or wholly synthetic introns were introduced at various locations in the ß-globin-coding sequence (59). These studies reinforced our earlier conclusion that splicing is critical for some step in the transcription or maturation process other than to produce a translatable sequence.

Another valuable feature of the pSV2 constructs was their use in transforming mammalian cells with genes that do not confer a selectable phenotype. Thus, pSV2 derivatives containing either *gpt* or *neo* could be cotransfected with DNAs whose encoded function did not allow for their selection or direct detection. Alternatively, genes could be inserted at any of several restriction sites in the pBR322 DNA segment, and their incorporation into cellular genomes is followed by coacquisition of the linked *gpt* or *neo* genes (69, 70). For example, human lymphoid cells transformed with a modified pSV2*gpt* containing a rearranged human κ light-chain gene in the pBR322 segment yielded cells with the GPT phenotype expressing the human κ light chain and the corresponding mature immunoglobulin (71). pMGD2*neo*, a useful variant of the pSV2 plasmid, was developed by Max Gassmann, a postdoc, and Greg Donoho, a graduate student, because it can be maintained as an episome in mouse cells (72). In addition to an expressible *neo* gene for selection, pMGD2*neo* contains the polyoma DNA segment containing the promoter/enhancer/ori and the sequence encoding polyoma large T protein. Consequently, foreign DNA sequences inserted into the pBR322 segment of pMGD2*neo* can be maintained in an episomal state in mouse embryonic stem cells.

Our purpose in developing transducing and transforming vectors was to introduce new genes into mammalian cells. This presumed that the means for their introduction into cells would be available when pure mammalian genes would eventually become obtainable. At the time SV40λ*dvgal* was developed, pure mammalian genes were unavailable, and methods for converting abundant mRNAs into cDNAs were devised later. Because full-length cDNAs were scarce and difficult to produce, Hiroto Okayama, a postdoc, set out to overcome this deficiency. He relied on base pairing of the mRNA's 3′ poly(A) terminus to a poly(T) tail that had been added to one 3′ end of a linear plasmid so that the cDNA formed by reverse transcription remained covalently linked to the cloning plasmid (73). This procedure was highly successful in obtaining a variety of full-length cDNAs; in our lab, Lars Thelander, a postdoc, obtained the cDNAs encoding the M1 and M2 subunits of mouse ribonucleotide reductase (74). Okayama subsequently modified the procedure so that the newly produced cDNA is flanked at the 5′ end by the SV40 early region promoter and a synthetic intron and at the 3′ end by a functional polyadenylation signal (75). This approach made it possible to isolate cloned cDNAs for which hybridization

probes were unavailable, but whose real or suspected functions could be detected or selected (76).

TARGETING GENES TO MAMMALIAN CHROMOSOMES

The expression of cloned mammalian genes and their modified derivatives is most often measured by the amount of mRNA or protein products formed after transfection of the DNA into appropriate cells. However, following transfection, the DNA is generally extrachromosomal and at nonphysiologic concentrations, and therefore, its activity may not reflect the expression of a chromosomal gene. Furthermore, because the transfected genes of most stable transformants are integrated at multiple ectopic chromosomal sites, their expression characteristics are unlikely to reflect the true activity of the resident gene. Accordingly, we sought to target newly introduced genes to specific chromosomal loci and, even more ambitiously, to replace endogenous chromosomal segments with altered versions.

Our first aim was to compare the frequencies of homologous and nonhomologous recombination events in mammalian cells. Subramani, using specialized plasmids as the recombination substrate, assessed the relative frequencies and specific products of intramolecular homologous and nonhomologous recombination (77). From these experiments, we learned that the frequency of nonhomologous recombination was considerably greater than the frequency of the homologous event; we also learned that the frequency of both modes of recombination was unaffected whether or not the test plasmid was replicating. To monitor recombination at chromosomal sites, Andrew Smith, a postdoc, established that recombination between two closely linked but differently mutated *neo* alleles, integrated at a single chromosomal site, was readily measurable by the formation of G418-resistant cells. With that result in hand, Smith determined that recombination also occurred between a single integrated mutated *neo* allele and a differently mutated *neo* gene introduced by transfection (78).

In a somewhat different experimental design, Maria Jasin, a postdoc, showed that targeted integration of a transfected DNA can be efficiently measured if as a result of the recombination the genomic target provides an essential element for the expression of an otherwise nonfunctional transfected gene (79). In her experiment, the recombination event provided a functional enhancer from an integrated SV40 early region to a transfected *gpt* gene lacking the promoter/enhancer. Importantly, she found that the frequency of recombination was greatly increased if the ends of the transfecting DNA were within the shared sequence homology of the integrated and transfecting DNAs, a finding reminiscent of yeast homologous recombination.

In all of the experiments cited above, the frequency of homologous recombination was low, of the order of 10^{-5}. Seeking to increase the efficiency of recombination between a transfected plasmid and a chromosomal site, Donoho, working with Jasin, devised a way to introduce double-strand breaks at a specific chromosomal target to achieve that end (80). In their experiment, an expressible *neo* gene was inserted into the single *hprt* gene (hypoxanthine phosphoribosyltransferase) of XY embryonic stem cells; such modified cells are resistant to G418 and to 6-thioguanine, the latter indicative of an inactive *hprt* gene. Importantly, the integrated *neo* gene was flanked at either or both ends by an 18-bp nonpalindromic sequence that is cleaved by the I-SceI endonuclease from *Saccharomyces cerevisiae* mitochondria. Comparable insertions of the *neo* gene lacking the I-SceI sites provided a measure of recombination in the absence of double-strand breaks. Cells were then cotransfected with a plasmid that expressed the I-SceI endonuclease and a separate plasmid containing a DNA segment that spanned the interrupted part of the *hprt* sequence. Resistance to aminopterin in the presence of hypoxantine and thymidine and loss of G418 resistance served as a measure of

recombinational repair of the *hprt* allelle. Their results revealed that introducing a double-strand break in the chromosomal target increased the frequency of homologous recombination 5000-fold. Using polymorphisms introduced into the transfected *hprt* sequence, they concluded that the repair of the *hprt's* missing sequence occurred by copying from the transfected DNA. This approach provides a way to create specific mutations in a gene at its normal chromosomal location.

Given that double-strand breaks appear to be readily repaired by homologous recombination in mammalian cells, we sought to learn more about the mechanism of this process. One approach was to examine this process in vitro. pSV2*neo* with gaps or deletions of various lengths in the *neo* sequence served as one of the substrates (the recipient), and a *neo* DNA that spanned the gaps or deletions in the *neo* sequence provided the donor substrate. Using a polymerase chain reaction with appropriate primers flanking the gaps or deletions in the *neo* gene, Rolf Jessberger, a postdoc, obtained a partially purified enzyme complex from mammalian nuclei that could restore the missing sequences in the pSV2*neo* sequence (81). Gaps and deletions were repaired with about equal efficiency and occurred by homologous recombination involving crossover and noncrossover events. The enzyme complex had an apparent molecular mass of 550–600 kDa and contained DNA polymerase ε, DNA ligase III, a 5′ to 3′ exonuclease, and several unidentified proteins (82).

EXPLORING HOMOLOGOUS RECOMBINATION IN YEAST

Considering that the frequency of homologous recombination in mammalian cells was markedly stimulated by double-strand breaks in DNA and that homologous recombination in *S. cerevisiae* also is dependent on double-strand breaks, we turned to a study of the proteins involved in repair of double-strand breaks in yeast. Initially, Montserrat Elias-Arnanz, a postdoc, and Antoine Firmenich, a graduate student, identified mutants that were unable to repair double-strand breaks introduced at an HO chromosomal site created by the expression of an inducible HO endonuclease (83). Most of the mutants they obtained were new alleles of the *Rad52* epistasis group, genes that had previously been found by their X-ray sensitive phenotype. One mutant, previously unknown, encodes the large subunit of the heterotrimeric single-strand DNA-binding protein, RPA (84). Thus, aside from being implicated in DNA replication, RPA is also essential for the repair of double-strand breaks.

Sharon Hays, a graduate student, found that the mutation affecting RPA was suppressible by overexpression of the RAD51 protein, suggesting that the two proteins might interact, a possibility that was supported using the two-hybrid technology (85). She surmised that the two proteins might be part of a complex that stabilizes the single strands at the DNA's break site early in the repair process. Hays also observed that Rad55 and Rad57 mutations, each of which impairs the repair of X-ray-induced double-strand breaks, were suppressed by overexpression of both RAD51 and RAD52 proteins. The two-hybrid assay also indicated that the RAD55 and RAD57 proteins form a complex and that the RAD51 protein interacts with RAD55 protein but not with RAD57. Because RAD51 and RAD52 proteins interact, we concluded that the repair of double-strand breaks in DNA involves a multiprotein complex in which a RAD51-RAD52-RPA complex associated with the RAD55-RAD57 complex is very likely to be involved in the repair process, perhaps in the initial phase of associating with the DNA ends (86).

At the time, only the RAD51 protein was known to catalyze a strand exchange between a single and double strand of DNA, ostensibly the initial step in the repair process. Using purified RAD51 protein, Eugenie Namsaraev, a postdoc, established that the strand exchange required both RPA and ATP hydrolysis and could be initiated either by an overhanging

3′ or 5′ end of the duplex DNA. Furthermore, he found that the ensuing branch migration needed to complete the exchange proceeds in either direction and is blocked if a sequence longer than 6 bp interrupts the homology between the single- and double-strand DNAs (87–89).

PUBLIC POLICY AND THE ASILOMAR CONFERENCE

After closing my lab, I was increasingly urged to engage in public policy issues related to biomedical science perhaps because involvement in such activities was not a new experience for me. I had my "baptism of fire" in that arena nearly 35 years ago with the debate about the recombinant DNA technology. That issue arose while our work on the construction of the SV40λ*dvgal* recombinant was under way. While one of my graduate students, Janet Mertz, was attending a course on animal cell culture techniques at Cold Spring Harbor, she discussed some of the experiments we planned to do when the recombinant was available. One in particular, propagating SV40λ*dvgal* in *E. coli* aroused the ire of Robert Pollack, the instructor in the course. In a rather heated telephone conversation, Pollock accused me of pursuing extremely reckless and dangerous experiments. He was concerned that these experiments created a risk that the SV40 oncogene could be spread outside the laboratory and create a cancer contagion. My initial reaction was to dismiss that likelihood as fantasy; there were counterarguments that I felt made that possibility unlikely. Nevertheless, in discussions with many colleagues and tumor virus experts, I concluded that, although the probability of Pollack's scenario was extremely low, it was not zero. Consequently, our plans for testing the biological properties of the recombinant molecule in mammalian and bacterial cells were put on hold until we could better assess its health risks.

Having raised the issue of the possible risks of working with SV40, as well as with other tumor viruses, Pollack and I helped organize a conference of virologists during January 1973 at the Asilomar Conference Center in Pacific Grove, California. The agenda for that meeting was to assess the potential risks inherent in working with animal tumor viruses in the laboratory and recommend ways to mitigate those risks. As a result of the conference's discussions, the NIH issued advisory guidelines informing scientists working with tumor viruses or other microbial pathogens of ways to limit the dangers inherent in their use.

A year or so later, the issue of risks associated with recombinant DNA experimentation surfaced in another context. Mertz and a colleague, Ronald Davis, discovered that the fragments produced by *EcoRI* endonuclease cleavage of double-strand DNA have single-strand overhangs at their 5′ ends. More significantly, they demonstrated that the ends were complementary and therefore capable of annealing to each other; more to the point, DNAs containing such ends could be joined together in vitro by the action of DNA ligase. Thus, circular molecules were formed by intramolecular "end joining" and long linear molecules were formed by end-to-end joining. The sequence of the so-called cohesive or sticky ends created by cleavages with *Eco*RI was shown by Hedgpeth et al. to be 5′-AATT, consistent with Mertz and Davis' estimate of a four-base AT-rich overhang.

Being aware of the Mertz and Davis finding that DNAs bearing cohesive ends generated by *Eco*RI endonuclease could be covalently recombined in vitro, Stanley Cohen at Stanford and Herbert Boyer at the University of California, San Francisco, constructed a variety of recombinant bacterial plasmids. More significantly, they showed that such recombined plasmids could be introduced and propagated in appropriate bacteria. These and related experiments were reported at the 1973 Gordon Conference on Nucleic Acids. The scientists in attendance were enthusiastic about the scientific implications of this new capability, but there were some who expressed concern about the inadvertent or intentional

misuse of the ability to create novel recombinant DNAs. That concern became public through the publication in *Science* magazine of a letter from Maxine Singer and Dieter Söll, the cochairpersons of the conference, to Philip Handler, the President of the National Academy of Sciences (90). That letter called upon the Academy to "establish a study committee to consider the problem and to recommend specific actions or guidelines should that seem appropriate."

Having learned that I had confronted the potential risks of recombinant DNA experimentation a year earlier, President Handler urged me to organize a small group of scientists to advise the Academy on how to respond to the requests of the Gordon Conference attendees. Before that committee was assembled, there was a startling development. Morrow et al. reported the construction and cloning of recombinants in which ribosomal DNA fragments from *Xenopus laevis* had been inserted into the pSC101 bacterial plasmid. This was the first demonstration that interspecies recombinant DNAs could be constructed and cloned. More than the earlier exclusively bacterial recombinants, the new experiments foreshadowed the creation and cloning of recombinants containing DNA from virtually any organism on the planet. It was that possibility and its implications that confronted the Academy's advisory committee when it met in April of 1974. Indeed, Cohen had informed us that he had received numerous requests for the plasmid from other investigators eager to create their own favorite recombinant DNAs.

Throughout the committee's deliberations it was clear that there were no concrete data concerning health risks attributable to recombinant DNA experimentation. Nevertheless, there were also no data absolving the planned experiments of any risk. Given the committee's lack of expertise in assessing the nature and magnitude of the risks, it recommended that a meeting of relevant scientists and public health experts be convened to make such an assessment and to recommend how the science could proceed. In the interim, the committee thought it prudent to alert the scientific community to the potential concerns and to urge that certain experiments be deferred until their potential risks could be evaluated. More specifically, scientists were urged to defer the construction of recombinants that would enhance the virulence, antibiotic resistance, and capacity for toxin formation in bacteria that lacked those capabilities. Considering the existence of oncogenes in mammalian DNA, investigators were urged to weigh carefully the construction and propagation of recombinants containing mammalian DNAs. Those recommendations were transmitted via letter to the President of the Academy and subsequently published in the *Science* and *Nature* magazines and in the *Proceedings of the National Academy of Sciences* (91). In spite of widespread consternation among many scientists about the proscriptions, the validity of the concerns, and the manner in which they were announced, the so-called moratorium was universally observed.

I served as the chairman of the organizing committee for the conference, held in February 1975 at the Asilomar Conference Center in Pacific Grove, California. The attendees included scientists from throughout the world, lawyers, journalists, and government officials. The principal aim of the meeting was to examine the state of the technology and its potential uses for research. Further, in its conclusions, the conferees were to recommend whether to lift the voluntary moratorium and, if so, under what conditions the research could proceed safely. After review and considerable debate regarding the limited data concerning the potential risks, the conference concluded, not without outspoken opposition from some of its more notable participants, that recombinant DNA research should proceed but under strict government guidelines (92). Such guidelines were subsequently promulgated by the NIH and by comparable bodies in other countries.

Looking back, the Asilomar Conference marked the beginning of an exceptional era

for science and for the public discussion of science policy. Its success permitted the then contentious technology of recombinant DNA to emerge and flourish. The use of recombinant DNA technology now dominates research in biology. It has altered both the way questions are formulated and the way solutions are sought. Without the tools of recombinant DNA, there would be no human or any other genome sequence. Equally profound is the influence it has had in many related fields. Even a brief look at journals in such diverse fields as chemistry, evolutionary biology, paleontology, anthropology, linguistics, psychology, medicine, plant science, and, surprisingly, forensics, information theory, and computer science shows the pervasive influence of this new paradigm. Additional testimony to the conference's success are the frequent calls to resurrect the "Asilomar Process" to resolve the ethical dilemmas posed by newly emerging ideas and technologies, most recently human embryonic stem cell research. Whether the Asilomar Conference is an appropriate model for resolving current conflicts of science and public policy is problematic.

THE AFTERLIFE

Closing my lab in 2000 was a very difficult decision. Although "carrying the ice bucket," i.e., doing experiments myself, had long since ended, I knew that I would miss the challenges that experimental science presents. Even more, I would miss the joy of watching graduate students grow and mature as they gained the assurance to function independently. But, I also conceded that it was time for the next generation to make their mark, and my department lost no time in recruiting several extraordinary young investigators to fill the slot I vacated.

However, I was not yet ready for the rocking chair, and there were other kinds of activities to challenge my intellect and energies. One was participation as a scientific advisor in the expanding biotechnology world. Initially, it was with DNAX (now named Schering-Plough BioPharma), then with Burrill and Company, an investment company, and more recently with Perlegen Sciences. Among the more engaging ventures has been serving on the Boards of Directors of Affymetrix and Gilead Sciences. In each of these endeavors, I learned quickly that the problems of advancing science to serve unmet medical needs are complex and challenging.

Quite apart from my activities in the laboratory, I had the great pleasure of working with Maxine Singer in a number of writing and other projects. At the time we began collaborating, she was a research biochemist at the National Cancer Institute where her work with polynucleotide phosphorylase contributed significantly to deciphering the genetic code. In time, she became Chief of the Cancer Institute's Laboratory of Biochemistry and carried out her seminal work identifying and characterizing the function of the repetitive sequences (SINES and LINES) in mammalian DNA. Following the Asilomar Conference on recombinant DNA in 1975, she was a principal in designing, drafting, and implementing the NIH Guidelines for work in this budding field. In 1988, Singer was selected to become President of the Carnegie Institution of Washington, a position she held for more than 10 years.

During the early 1980s, we had many conversations about whether the time was ripe to write a textbook that would enable students to understand the science and implications of recombinant DNA technology. Having decided that it could serve that purpose, we wrote *Genes and Genomes: A Changing Perspective* (93) and were gratified by the feedback we got from students round the world. Believing also that there was a need to inform nonscientists of the nature and implications of the "genetic revolution," we wrote a follow-up book *Dealing with Genes: The Language of Heredity* (94). Believing that it was also important to focus

in greater detail on areas of biology that had been significantly impacted by the recombinant DNA technology, we edited a collection of articles by experts in their respective fields for a volume entitled *Exploring Genetic Mechanisms* (95).

Emboldened by the moderate success of these ventures and our concern that students of biochemistry and genetics were grossly ignorant of the founders and pioneers of their fields, Singer and I undertook the biography of George Beadle. Beadle emerged as a leading scientist—the one gene-one enzyme formulation of gene function was the accomplishment that earned him and his colleague Edward Tatum the 1958 Nobel Prize in Medicine. The book, *George Beadle: An Uncommon Farmer* (96), placed Beadle's far ranging work in the context of the emergence of genetics in the first half of the twentieth century, what some refer to as the classical period. Unexpectedly, Singer and I found that the five years of research on Beadle's life and times were as challenging and gratifying as any laboratory investigations we had done earlier.

EPILOGUE

Whether it is the creation of a painting, a work of poetry or music, or uncovering the molecular secrets of cell division, each integrates the capacity to wonder, to imagine, to explore, and to create, attributes we generally label curiosity. But curiosity is best served by what Medawar referred to as "the passion for discovery" and a "rage to know."

J. Robert Oppenheimer expressed that sense in another way: "Although we are sure not to know everything and rather likely not to know very much, we can know anything that is known to man, and may, with luck and sweat, even find out some things that have not been known to man before."

For me, from as far back as high school to today, finding out something that has not been known before produces exhilarating experiences. Such experiences are rare, personally rewarding, and not always recognized by prominent prizes. Triumphs of the intellect and the success of discovery or creation have their own prize—self-satisfaction. That feeling may be the principal attraction that draws scientists on.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

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