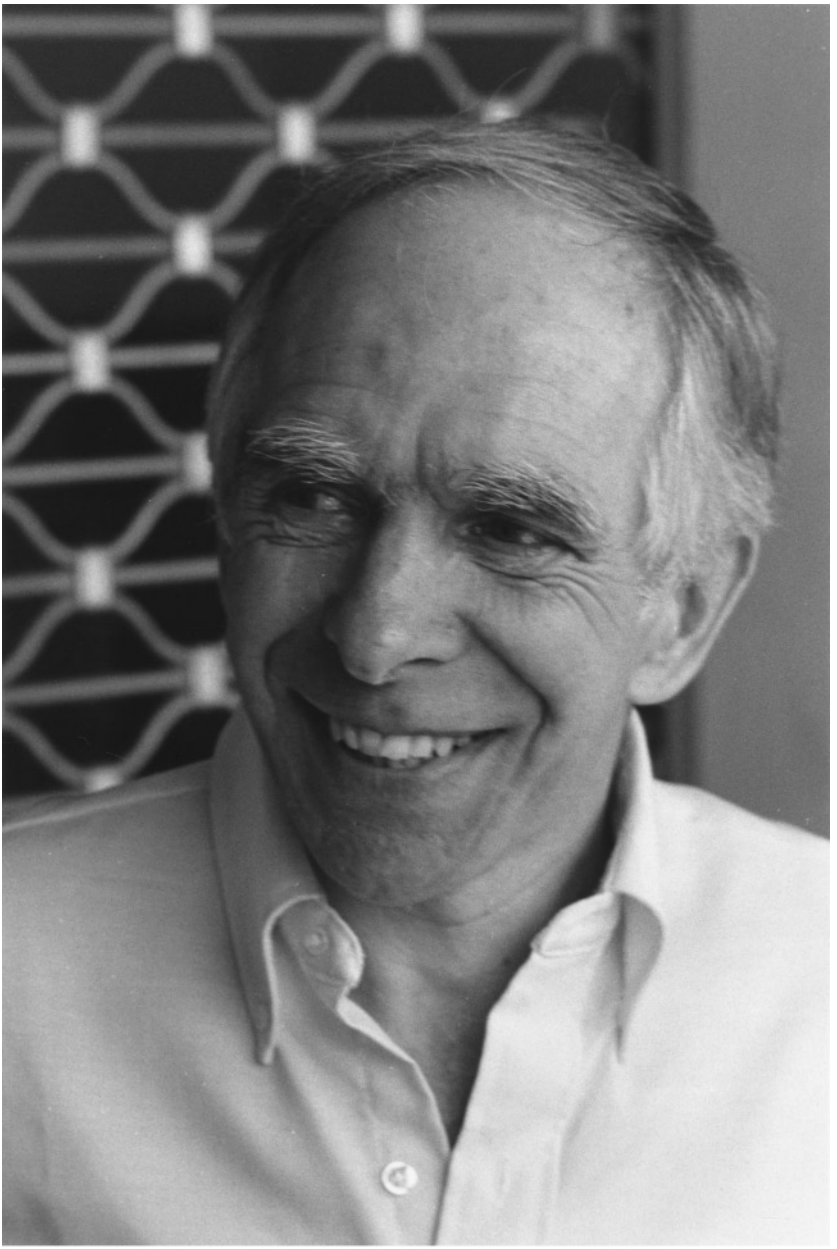

Julian Davies

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GATHERING NO MOSS

Julian Davies
Department of Microbiology and Immunology, University of British Columbia, Vancouver British Columbia, Canada V6T 1Z3; email: jed@interchange.ubc.ca

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■ **Abstract** I never imagined that I would be asked to write an autobiography in a microbiology tome. For that matter, little did I think that I would consider microbiology the most intriguing subject in the life sciences and the only field I wanted to study. My formal scientific training was in chemistry. This is a recounting of my conversion and the opportunities I have had to work in the microbial sciences with some of the major figures (and characters) during a period of marvelous intensity and productivity. I want to recognize and thank my many distinguished colleagues for the ways in which they have helped me to experience a fruitful and stimulating life as a microbiologist.

CONTENTS

GROWING UP

I am proud of having been born in Wales, but I am not nationalistic; the only manifestation of my origins is my support of the Welsh rugby team (especially when they are playing the historic invader, England) and a life-long ambition to

play for Wales. My mother and father had a tobacconist/barber shop near Swansea; I remember little of this, as we left when I was five years old for a suburb of London to be near my mother's family. My life as a rolling stone had begun.

In 1939 we moved to Kent, where my father had obtained a job in the Chatham Naval Dockyard. He was a shipwright and carpenter by training, with a deep love of the sea. Then the Second World War began, and I was "evacuated" with my sister and all the other children to a supposedly safe area elsewhere in the county. With the typical wisdom and forethought of the authorities, we were billeted with families near a major Royal Air Force base that soon came under serious air attacks, so we were taken back to Chatham, where school seemed to be a perpetual test of our ability to troop to the air-raid shelter in an orderly manner and wear gas masks for extended periods in class. Sporadic bombing of the dock area had started and we were under constant blackout. In 1940 when the Battle of Britain began and Chatham was again deemed hazardous, we were shipped by train to Wales and billeted with families in small mining towns in the valleys. I quickly learned about the rigors of life in a mining community; the father of the family with whom I stayed had been killed in a mine accident just before I arrived in Llanharan and the oldest son perished in a rockfall shortly thereafter. Education seemed to play little role in my life there. I spent a lot of time larking about with the lads and learned how to whistle piercingly through my fingers, but the only science I practiced in this period was building dams in streams in the woods so that we could make muddy pools for swimming. My sister was billeted in a village about eight miles away. I saw her occasionally, but our parents managed only a few visits.

By 1942 the air raids had calmed down and we returned home to get on with schooling, which was now focused on passing the "11-plus," an examination required for entry into the grammar school system. It was about this time that I manifested an interest in science—specifically chemistry—for no particular reason that I am aware of. Gradually, I built up a decent home chemistry set with glassware and chemicals that I bought with my pocket money from a local chemist's shop (pharmacy) or by mail; it seemed that one could buy almost anything one wanted—there were no restrictions on poisons, for example. I had no idea what I was doing, but I enjoyed creating dramatic color changes and mixing potassium nitrate and sulfur to make fireworks, the louder the better. So far as the war was concerned, rather than worrying about bombs, my mates and I concentrated on spotting planes, collecting choice bits of shrapnel, and asking the Yanks massing in Kent before D-Day, "Got any gum, chum?"

I passed my 11-plus and started at the grammar school, which was recognized in the county for its high educational standards. The Headmaster was a fearsome man and (I realize in hindsight) a masochist. Punishment, usually a caning or "500-lines" (some inane sentence written legibly 500 times in after-school detention), was normally administered by teachers. Major crimes (like going in the air-raid shelters when there was no raid on) were the Headmaster's purview. He had a collection of canes in a cupboard in his office and would take his time choosing the one with the correct whippiness before administering "six-of-the-best."

The words "Bend over, boy" were the fateful prelude. The marks of the cane lasted several days and were inevitably exposed at home on bath night—my parents usually commented that I must have deserved it.

In 1944 we moved to southwest Wales for family reasons and there I had the time of my life. We had never lived in one town for so long! My sister and I attended a first-rate coed school and I had lots of good friends, albeit we existed in a competitive environment; four or five of us always vied for "top of the class." My performance in classical subjects was mediocre, but I competed well in chemistry, physics, and mathematics. In these courses I had excellent teachers who were keen to see their students do well, and my interest in organic chemistry was fanned by the Headmaster of the school, an Oxford chemistry graduate. The structure of penicillin, the first complex organic structure I ever committed to memory, fascinated me and I liked to draw it out on paper. Another monitor and I had the job of preparing and cleaning up after chemistry demonstrations, which gave us free run of the lab on Saturday mornings. In addition to routine preparations, we took the opportunity to try out all sorts of reactions, some of them more violent than expected, but causing no damage more serious than an occasional burn.

Life outside of school was also good; the war had ended and although chocolate was still rationed, there were fewer restrictions. I became active in sports (twice disqualified for running in a walking race!) and performed in a number of the productions of the local playhouse, which may have helped me later in giving lectures and seminars. I am always a little nervous before any presentation in public, but once on my feet, I have no fear.

Entry to university involved two national examinations. At 16 I passed School Certificate (including French, which amazed my teacher and came as a great relief to me!) and moved into the rarified atmosphere of small classes and special assignments in preparation for the Higher School Certificate, which I took in 1950, winning a national scholarship and admission to almost any university in the country. The Headmaster was insistent that I go to Oxford, but I refused, since that would have meant a test in Latin—I could not face another foreign language. A friend recommended Nottingham University, where he had received his B.Sc. in Chemistry. So I visited, was offered a place, and left Milford Haven to embark on my university training. I still had my chemistry set.

UNIVERSITY DAYS

My three years of undergraduate training at Nottingham were typical university years. A lot of time was dissipated on nonessential activities, but I learned that I really did want to do chemistry. The University Chemistry Society organized occasional visits to companies in the area, but as I recall, the main benefit of these trips was the meal in the factory canteen (much better than in digs!) and the raucous bus ride home with several stops at pubs. Academically speaking, I did quite well at Nottingham, in mathematics as well as in chemistry. (I would have fared better

in the chemistry final had I been able to remember all the reactions that could take place at a methylene next to a carbonyl!)

Deciding to go on for a Ph.D. and remembering my fascination with penicillin, I asked a professor who worked on microbial products if he would take me on. He was diligent and helpful and gave me free rein in my approach to the project he suggested, which was the total synthesis of a fungal naphthoquinone called flaviolin in order to confirm a proposed structure. (In those days all structures were confirmed by synthesis, since infrared, NMR, and mass spectroscopy were not generally available.) Things went well (apart from a setback when the dessicator holding two-months worth of a precious key intermediate was knocked off the bench by a highly apologetic cleaning lady), and the structural identity of the synthesized and natural product was confirmed. We were excited about this, only to learn that A.J. Birch, a well-known Australian chemist, had completed the synthesis of flaviolin by a "more biological" route! Scooped on my first research! Anyway, the work was published (10) and I looked for another project to finish my thesis. I wanted to work on a natural compound of unknown structure and chose sterigmatocystin, a fungal metabolite that had been isolated some years earlier. It was not known at that time that sterigmatocystin and flaviolin are polyketides; I was working on a large and very important class of secondary metabolites that includes many important antibiotics. Nor did I know that sterigmatocystin is a close relative of the highly mutagenic compound, aflatoxin! Not that this made much difference, since everyone in the lab used most organic reagents with abandon and employed grams of nitrosoguanidine and dimethyl sulphate on a regular basis! I isolated lots of sterigmatocystin and weighed all these chemicals on an open balance. It was common practice to wear a necktie, which was part formality and part having somewhere to wipe a spatula before weighing something else. As far as I am aware, all students in the lab survived, which suggests that natural product chemists must have excellent DNA repair systems!

Life in the chemistry research lab at Nottingham was hard work but very enjoyable. We put in long hours in the lab but also had our good times together, rugby and cricket matches, pubs and dances. I had several close friends and shared digs with them now and then (I moved five times, but only once was I actually thrown out.) Studies on the chemical degradation of sterigmatocystin went well, and I wrote my thesis in 1956 with a proposed structure based almost entirely on chemical modification and degradation (11). I did have one infrared spectrum (which was not much help, since interpretation of such information was primitive) and it was included in my thesis, a first at Nottingham. At this point Alan Johnson, the Head of Department, suggested postdoctoral study to the United States, and when I expressed an interest in continuing work on natural products, he wrote to Gilbert Stork at Columbia University in New York. Imagine my thrill when I received an offer, which I accepted immediately. My mother and father encouraged me fully in my desire to get a Ph.D. and then to postdoc in the United States. Unlike numerous aunts and uncles who often asked what I was going to do when I grew up, my parents never posed this question.

AN INNOCENT ABROAD

I sailed from Southampton in early September of 1956. The arrival in New York Harbor was an awesome experience, as was meeting New Yorkers, who were just like the movies. The biggest shock was the Stork laboratory, where the graduate students were knowledgeable and aggressive! Despite my Ph.D., I felt inferior! They thought differently about chemistry, analyzing all reactions electronically and covering the blackboards with arrows. It was the beginning of a great learning experience—being thrown into a world-class laboratory in a leading U.S. university and having to participate in intense lab meetings and seminars. There were several other foreign postdocs, among them the late Willie Parker, who became a great friend; we provided the main axis for American-baiting, admittedly receiving as good as we gave. In addition to lab work, there was Monday night bowling and intramural softball—and baseball. The lab was split between Yankee fans from the Bronx and Dodger fans from Brooklyn; when Don Larsen pitched a no-hitter for the Yankees in the World Series, you'd have thought the world had ended. Lunch was also a new experience—every day we walked a couple of blocks to Adolph's Deli and I learned about hot pastrami and chopped liver on rye. An introduction to pizza followed.

Working for Gilbert was a revelation. He was unlike any "Prof" I had ever met and he taught me a great deal about doing research. In our lab meetings we were encouraged to express our own ideas, and Gilbert, who was a mercurial thinker, always came up with something better. I gained knowledge of another class of natural products, the terpenoids and steroids, and worked successfully on the total synthesis of a pentacyclic triterpene, β-amyrin (18), winning a race to complete the synthesis against R.B. Woodward and A. Eschenmoser, two great organic chemists of the day.

I had arranged to stay at International House on Riverside Drive, close to the university, and this was the center of social activities outside the lab. It was stimulating to encounter so many people of different nationalities, working in other disciplines. It was there I met Dottie, the woman who became my wife. There is no doubt that my first year in the United States was profitable!

Gilbert arranged a postdoc for me at the University of Wisconsin (UW) when I completed the β-amyrin synthesis. In retrospect this move may have been a mistake, given how well my work at Columbia was going, but I wanted to try another aspect of organic chemistry. The experience in Stork's lab and in New York gave me great respect for the vitality of U.S. science; I left a more mature and confident scientist, my exposure to colleagues from Brooklyn and the Bronx having prepared me for anything. I never had second thoughts about a career in organic chemistry at that time.

Dottie and I were married in December 1957 in Waltham, Massachusetts, and took a mobile honeymoon on route to Wisconsin—via Niagara Falls, of course. The 18 months in Eugene van Tamelen's lab were rewarding in a personal sense, although work on the synthesis of an alkaloid was not so successful. Dottie and I

integrated well into the community and made good and lasting friendships in the Chemistry Department and in Madison.

BACK TO BRITAIN

Feeling that I shouldn't desert the country that had given me my education, I applied to several universities in the United Kingdom and was hired without an interview to the Department of Chemistry at the Manchester College of Science and Technology. At this time I assumed that I would settle into an academic career in organic chemistry in Britain and went there in September 1959 with a wife, daughter, and great expectations.

We found a flat in a delightful old village in Cheshire and settled down to enjoy life in England. I took the train to Manchester every day, my first experience of commuting. At that time, Manchester was very sooty and I soon learned that a white shirt became light gray by the end of the day! There were occasional "inversions" when the desks, floors, and chairs were covered in grime, but that was part of life and the least of my problems with the Department of Chemistry. The Head was extremely autocratic with a large research group that included most of the faculty and was focused on fluorine chemistry; I made several good friends there but was an outsider with research goals different from the others. To me natural products offered many challenges in isolation, structure, and synthetic chemistry, and I worked hard to set up a program in this area with two Ph.D. students. However, not long after arriving in Manchester I became interested in biosynthesis, and in late 1960, when a group of students asked me if I would give some lectures in biological chemistry, I agreed. Thus began my career as a teacher of subjects about which I knew nothing. Gradually the enjoyment of reading about the basic biochemistry of the cell and teaching it, combined with the growing dissatisfaction of being alone among a large number of "fluorine types," made me realize that chemistry was losing its flavor; the biology of natural products became increasingly attractive.

The event that ensured the transition was meeting the well-known microbiologist Milton Salton, who told me about his work on bacterial cell walls and invited me to do some work in his lab. After teaching me how to grow bacteria and use a centrifuge, Milton set me to isolating and characterizing intracellular bacterial structures called mesosomes. Electron micrographs allowed us to track these onion-like structures, which were later shown to be artifacts of preparation, meaning that my first citation in the microbiological literature was for a nonexistent structure! However, I was hooked on the subject and ready to take steps to become a microbiologist. Milton asked me with whom I would like to work, and given my interest in biosynthesis, I suggested Bernard Davis or Charles Yanofsky, who were working on aromatic amino acids. Milton contacted Bernie, and I received an offer to join his lab at Harvard Medical School.

SCIENCE IN BEANTOWN

Misreading at Harvard Medical School

Dottie and I (and two children) arrived in Boston in August 1962 and I began work in the Bacteriology and Immunology Department. Among other things, Bernie was studying the mode of action of streptomycin, convinced that this antibiotic killed cells by disrupting bacterial cell membranes; he was unhappy that Roger Stanier and Charles Potts had just published a very convincing argument in favor of the ribosome being the drug target. This had been suggested earlier by Agnès Ullmann (of cyclic AMP fame) and confirmed by work from Joel Flaks in Philadelphia, who showed inhibition of ribosome function by streptomycin in a cell-free system. Bernie did not believe these results and asked me to repeat the experiments using a poly U-translation system. I knew nothing about cell-free protein systems or streptomycin and its mode of action; in fact, I knew precious little about microbiology at all! This became horrifyingly obvious when I attended my first departmental seminar on phage T4 biology, given by Salvador Luria from MIT. I understood not one word of the talk (partly because of Salva's Italian-American accent) and wondered what I was getting into. However, Bernie enlisted the help of Mahlon Hoagland, who was experienced in protein synthesis and gave me lots of good advice. With some painstaking lab instruction from a number of people, including Ron Hancock and Don Dubin (both in Bernie's lab), Harold Amos also at Harvard Medical School, and Pierre Spahr in Paul Doty's laboratory in the Harvard Biolabs, I got started on experiments. Pierre taught me how to make polyuridylic acid, which was not commercially available then, and I was able to set up an efficient cell-free system, but the results were not welcomed: It was clear that streptomycin inhibited protein synthesis. Polyphenylalanine production on ribosomes from sensitive strains was inhibited by low concentrations of the drug, but ribosomes isolated from an isogenic-resistant strain were unaffected. Bernie was not happy!

At this juncture, I had a bit of luck; Mike Cannon, a friend of mine from Manchester who was doing a postdoc in Wally Gilbert's lab, told me that Wally was isolating ribosome subunits to study tRNA binding. So I went over to the Biolabs to learn how to make *Escherichia coli* 30S and 50S ribosome subunits for in vitro translation, the idea being that mixing subunits from sensitive and resistant strains might show where the drug acted. These were overnight experiments: making the cell-free extracts and gradients at about 6 p.m., driving back after dinner to load the gradients in the centrifuge, and collecting the fractionated subunits the next morning to make hybrids for use in poly U-translation. The results were unambiguous: Streptomycin worked at the level of the 30S subunit. This was very exciting, and my first contribution to the field. I was by now meeting with Pierre Spahr and Wally occasionally to discuss experiments, and the idea of making radioactively labeled poly U to test if streptomycin affected the binding of mRNA to the 30S subunit was mooted. Once again the result was clear-cut: The antibiotic did not interfere with the binding of poly U to ribosomes.

One evening at a party in Cambridge I met Jim Watson, who asked me what I was doing and invited me to give a seminar at his lab meeting. After my seminar, Jim told me that I should publish my work soon; the results were important. When I explained that publication could be difficult because Bernie was convinced that streptomycin targeted the bacterial cell membrane, Jim said that he would take care of this. A week or so later Bernie told me that I should write up the work, that Jim would submit it to the *Proceedings of the National Academy of Sciences* (*PNAS*) (9), but that he, Bernie, would not put his name on the paper—what could I say?

About this time I became better acquainted with Luigi Gorini, a faculty member in the department who was a great character and an excellent scientist to boot. He was a true bacterial geneticist, best known at the time for his work on the regulation of arginine biosynthesis. Luigi had hair that stuck out all over the place and seemed to be constantly excited about something. When I first presented my work on the role of 30S ribosome subunits in streptomycin activity, Luigi took me aside to tell me about his "funny mutants." He had been isolating streptomycin-resistant mutants of arginine auxotrophs of *E. coli* and found that some of the resistant mutants would grow in the absence of arginine—the suppression did not require the presence of arginine, just the str^R mutation. He could not explain this and neither could I (I knew nothing about genetics), but we wondered if the fact that mutations to streptomycin resistance changed the 30S subunit had anything to do with it.

One day when Luigi, Wally, and I were discussing the funny mutants, Wally suggested that the accuracy of translation might be involved. So we tried experiments with cell-free systems in the presence and absence of streptomycin, using poly U as messenger and other amino acids in place of phenylalanine. To our great satisfaction, incorporation was stimulated in the presence of streptomycin. The effect was not seen when we used streptomycin-resistant ribosomes, whereas other aminoglycoside antibiotics stimulated translation equally well with streptomycin-sensitive and -resistant ribosomes. We found that leucine replaced phenylalanine in the presence of streptomycin, whereas neomycin stimulated the incorporation of several other amino acids. This experiment clearly demonstrated that streptomycin causes mistranslation by binding to the 30S subunit and that this subunit is critically involved in the decoding step during mRNA translation. It was an exhilarating time; every day we tried different variations of the experiments with interesting results, some of which could not be repeated, but the less said about that, the better!

Jim insisted that we publish immediately, so a manuscript was written and sent to *PNAS*. That's when trouble began! Bernie was away on leave in Mexico writing his textbook and knew nothing at all of this work. He and I had arranged to meet at Stanford before attending a small meeting on streptomycin at the University of California, Berkeley. I was asked to give a seminar in the Stanford Biochemistry Department: my first-ever public presentation in microbiology and I was quite apprehensive. I had already met Arthur Kornberg at dinner at his house, and before

my seminar Paul Berg asked me to give him a private review of my work. However, when I walked into the seminar room and faced Arthur Kornberg, Charlie Yanofsky, Paul Berg, the Lederbergs, Dale Kaiser, Dave Hogness, and Luca Cavalli-Sforza, it seemed like a trial by fire. But all went well; Josh Lederberg woke from sleep to ask questions and I managed to provoke a few laughs. Arthur Kornberg then surprised me with my first honorarium ($50); I didn't know one got paid for giving talks!

The meeting in Berkeley was arranged by Roger and Germaine Stanier, an extraordinarily kind and gracious couple. It was fun and our results were well received. Wally was there, and Gunther Stent with his postdoc, Charles Kurland. Bernie knew about the mistranslation work by this time but was not aware that a manuscript had been accepted for publication. During the meeting Wally and I gave Bernie a copy of the paper, which he returned to us with all kinds of corrections (he was a fiendish editor). When we told him that it was about to be published in *PNAS*, Bernie was furious and insisted that it be withdrawn. Eventually he agreed to publication as long as he could correct the galley proofs, which he did, severely (6).

After this Bernie left me on my own in the lab to work on miscoding by different antibiotics and to isolate resistant mutants with the expectation that they would indicate ribosome changes. I tried to identify altered ribosomal proteins in resistant mutants by gel electrophoresis. In these various ventures I had great help and good friendship from my excellent technician, Sue Armour; Monty Bissell, a medical student; and Porter Anderson (of pneumococcal vaccine fame). Porter did some nice work showing that the antibiotic spectinomycin also acted on the 30S subunit, and this was published in *Science* (5). The paper went through eight or nine drafts in the hands of Bernie, but we finally got back to where we started.

My three years at Harvard were marvelously instructive in many ways—interactions with colleagues in the department, such as Eliora Ron and Howard Goldfine, frequent visits to Jim's group in the Biolabs, and encounters with visiting scientists brought by Bernie to talk with us. The department lunches cooked by Mrs. Sullivan were always fun and often instructive. Hugh McDevitt taught me all the immunology I wanted to know in one lunchtime. Bernie was a vital influence at this stage in my career; he was strongly supportive and I owe him much—but he could be difficult! Probably the greatest influence came from Wally and Luigi, who were thinkers "outside the box." They demonstrated that research is best done by following one's hunches. Often these speculative ideas are not correct, but when they are—bingo! A key part of creativity lies in not worrying about being wrong (sometimes).

Another valuable experience at Harvard was as a teaching assistant in the laboratory course for medical students that was given in conjunction with Luigi's lectures on microbial genetics. I attended his lectures and supervised part of the lab course. At first I learned more than the students, and I often had difficulty in answering questions (not only because we had not understood Luigi). Usually my ploy was to ask if the student had looked it up in the library. Of course not, so I would suggest that we look it up together. I gained a great deal of practical microbiology experience at this time.

However, my life turned in another direction in 1964, when François Jacob was invited to give the Shattuck Lectures at Harvard. As I was driving him home from a dinner party at Jeanna Levinthal's house, he asked if I would like to work with him at the Pasteur Institute in Paris. What a question! I suspect that Bernie had surreptitiously set this up; at any rate he asked the National Institutes of Health (NIH) if my Career Development Award could be used in Paris. The answer was "yes" and we started planning for our new adventure.

Translation at UW

During my stay in the Boston area, the hottest topic in the life sciences was the unraveling of the genetic code: identifying the triplets responsible for the insertion of specific amino acids in protein synthesis. At first this was a competition between the NIH group (Nirenberg) and the New York University group (Ochoa), both working with randomly synthesized RNA heteropolymers, identifying the peptides synthesized and so making codon assignments. The results were often difficult to interpret when using random polymers, until Gobind Khorana's group at the University of Wisconsin entered the fray, using chemically designed polymers that permitted more accurate assignments (Figure 1). I wondered if mistranslation induced by aminoglycoside antibiotics could be due to effects on specific bases and asked Bernie if I could test this in Khorana's lab. Bernie proposed the idea

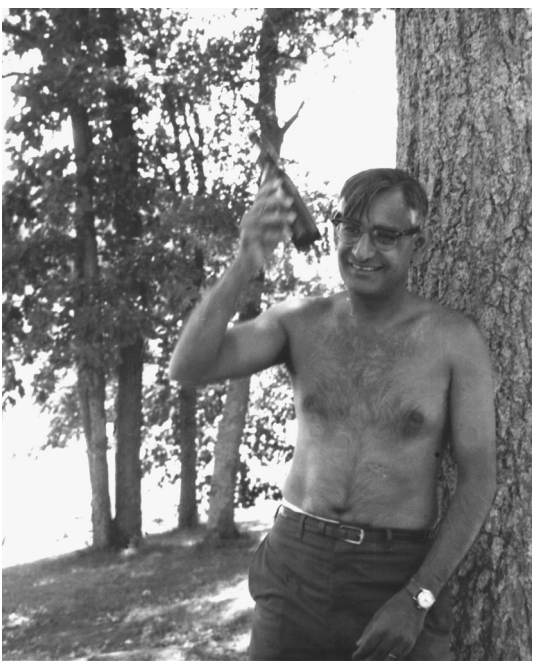

Figure 1 Gobind Khorana relaxing at a lab picnic.

to Gobind, who thought that I might be able to get results quickly, so I arranged to go to Madison in July 1965, leaving Dottie to prepare for the trip to Paris. The Enzyme Institute was another stimulating place to be, with new codon assignments being made and frequent seminars and group meetings that discussed all aspects of the genetic code, polynucleotide synthesis, and the like. I enjoyed the intensity and productivity of a big lab with the thrill of daily discoveries and formed good and lasting friendships with people like David Jones, Bob Wells, Vern Petkau, Hermann Bujard, Dieter Soll, and the late Richard Morgan. I collaborated with a postdoc, David Jones, who taught me the tricks of polynucleotide translation. We checked out all the available polymers for effects of streptomycin or neomycin on translation; I already knew that the latter antibiotic should be more active. The only limiting factor was the availability of radioactively labeled amino acids—and time! But we got convincing results very quickly and submitted a paper to the *Journal of Molecular Biology* proposing a set of rules for mRNA misreading on ribosomes in the presence of aminoglycosides (8) (Figure 2).

THE PASTEUR INSTITUTE

Learning About *lac*

In September 1965 we set off with three children for France. Fortunately, an apartment near the *Jardin du Luxembourg* had been arranged for us by the charming

Figure 2 Going-away party aboard the *Sylvania* in Boston Harbor: Luigi Gorini, Don Dubin, Harold Amos, Ann Farnham, Sue Armour.

Figure 3 Nobel Laureates 1965: André Lwoff, Jacques Monod, François Jacob.

Sarah Rapkine, who acted as the concierge for all visiting fellows at the Pasteur Institute and introduced us to great wines occasionally when she shared a couple of dozen bottles of a lesser vintage she had received from her friends, the Rothschilds.

Jacob's laboratory was under the eaves on the top floor of an old building, with a wonderful view over the rooftops to the *Tour Eiffel*. (My first French lesson was from Jacob's secretary, Gisèle, who took great pains to teach me how to pronounce *Rue du Docteur Roux* correctly!) On the same floor were Elie Wollman, André Lwoff, and Pierre Schaefer—quite an intellectual powerhouse! Luiz Pereira da Silva, Harvey Eisen, Yukinori Hirota, and Ethan Signer were also working with Jacob. I shared a lab with Ethan, who taught me a lot of the science and techniques of bacterial and phage genetics—it was there that I discovered another use for toothpicks. My knowledge of bacterial genetics was still limited, and I certainly had not appreciated how important genetics is to microbiology! My project was to obtain a detailed genetic map of the *i* and *o* regions of the *lac* operon, and all the mysteries of bacterial conjugation and phage P1 transduction were rapidly learned under the astute supervision of Ethan. The project was slow to begin but soon gathered momentum as knowledge of the various techniques and their use was acquired.

Then everything stopped! François Jacob, André Lwoff, and Jacques Monod were awarded the Nobel Prize in Medicine and Physiology (Figure 3). The labs were inundated with TV cables, cameras, and questioning reporters. There were a few exceptional receptions where I learned to appreciate good champagne and French canapés, but we were relieved when we could get back to science.

The mapping of the *lac i* and *o* genes was not an easy problem, since the mutations were closely linked and there was no simple selection method for wild-type recombinants. Several positive selections were tried, such as phenethyl-β-galactoside, which produced toxic phenylethanol in the presence of β-galactosidase, but there was too much spontaneous cleavage and cell killing. On one of his

visits to Pasteur, Melvin Cohen suggested that I try using 5-bromo-4-chloro-3-indolyl-β-D-galactoside (a.k.a. X-gal) as a means of screening for *lac* i^+ recombinants on plates. At the time this was a rare product that had been developed as a histochemical stain for use in mammalian cells. Initial results were very promising. By combining X-gal with the use of a *galE* recipient and recombinant selection on glucose-phenyl-β-D-galactoside, productive crosses were possible. Hundreds of crosses employing both conjugation and P1 transduction were carried out. Curiously, I was working with P1 on the fourth floor, while on the ground floor Maxime Schwartz was doing P1 transductions for *E. coli mal* operon mapping and strain construction. My transductions worked beautifully; Maxime's did not. Maxime did a transduction in my lab and it was successful. Had we discovered an effect of altitude on P1 transduction? We did not carry out complete controls (halfway up?) and just accepted it as one of those mysteries of experimental science, substantiating that "in a well-designed experiment with all parameters carefully controlled, the microbe does what it likes." In any event we could not blame it on the quality of the kit, as is often the case nowadays—there were none!

Sydney Brenner often visited Jacob's lab, and his amazing intellect, knowledge, and love of science was always invigorating (Figure 4). As everyone knows, Sydney loves to talk about the personalities in the field, and his endless collection of witty stories provides great enjoyment. François Jacob had been closely associated with "Sid-nay" since the well-known messenger RNA experiment, and they had collaborated on DNA replication studies. Theirs was a friendship bonded by mutual respect. In 1966 I was fortunate in that (*a*) Jacob asked me to stay another year and (*b*) the owners wanted their Paris apartment for the month of August, so (*c*) we spent the summer in Cambridge and I worked in Brenner's laboratory.

A Summer at MRC

Our time at the Medical Research Council (MRC) was short but stimulating. We rented Guido Tocchini-Valentini's flat, and with the loan of his bicycle I was able to get to the lab easily. I knew about Sydney's classical studies on frameshifting in bacteriophage T4 and wanted to test if streptomycin or streptomycin resistance might cause phenotypic suppression of bacteriophage T4 mutants by miscoding. The experiments were relatively easy, since they involved measuring burst sizes after infection under different conditions. Sydney was no longer interested in T4, having begun work with *Caenorhabditis elegans*, so Leslie Barnett, Sydney's long-time associate, patiently taught me about T4. I didn't observe much effect of antibiotics but found that certain streptomycin-resistant strains, especially those dependent on the drug, actively suppressed some of the T4rII frameshift mutants. This was a surprise and I wrote a note on the subject, but because the results could not be explained, it never saw the light of the day. (What we know about the ribosome now might explain this.) The MRC was an active laboratory with many visitors, including Harvey Lodish (who had a bench next to mine) and the late Paul Sigler. Fred Sanger, a modest and charming person, was making great advances

Figure 4 Sydney Brenner, a man of many talents.

in nucleic acid structure determination, and Francis Crick wandered in and out talking of the interesting work going on in the laboratories visited on his travels. Working with Sydney was a revelation; he has great ability in designing simple experiments to answer biological questions and writes papers like no one else. I hope that some of this rubbed off on me in the all-too-brief sojourn in Cambridge.

Then it was back to Paris in September, with a good collection of T4 phage and bacterial mutants and a significantly better understanding of bacteriophage genetics. However, *lac* operon mapping was still the main task and I was making good progress. Several of the operator mutants that I used were previously identified $i^- o^c$, but in crosses they mapped in the *lacI* gene. This led to their re-identification as a new class of repressor mutant, i^{-d}, partially dominant to wild type in both *cis* and *trans* orientations. Jacques Monod let me borrow a couple of the original lab notebooks on the condition that I keep them in the laboratory. Many operator (*o*) and repressor (*i*) mutants with a variety of phenotypes had been isolated and were

described in the pages written by Jacob and Monod. Several classes had varied responses to the inducer isopropyl-β-thiogalactoside, and I was able to pick out the i^{-d} type quite easily. It was clear that everything fit nicely with the operon theory.

Earlier in the summer of 1966 Yukinori Hirota had given me a bacterial strain carrying an R-factor (R100). The strain was resistant to several antibiotics, including streptomycin, but the mechanism of resistance to the latter was unknown. This aroused my curiosity, since I thought I knew all there was to know about streptomycin resistance. So I isolated the ribosomes from the R^- and R^+ strains and tested them for susceptibility to antibiotics using a poly U in vitro system—the two were indistinguishable! (If I hadn't purified the ribosomes in my zeal to do the experiment properly, I might have had a different result, especially since there was a marginal effect with switched supernatant fractions.)

I read the early literature on R-factors, with Yuki filling me in on a lot of aspects of the phenomenon, and realized that this would be a productive research topic for the future. The future? I had made no plans, assuming that I would return to Harvard Medical School after my stay in Paris, although I was only a research associate there. Then I received a letter from Hatch Echols inviting me to the University of Wisconsin to interview for an opening in the Biochemistry Department. The upshot of visits to Boston and Madison in the autumn of 1966 was the offer of a tenured position from the University of Wisconsin and ambiguity from Harvard Medical School. When Bernie said he saw no faculty position for me as long as Luigi was in the Department and Luigi said he saw no future for me as long as Bernie was in the Department, I decided to accept the offer from Madison.

We had mixed feelings about leaving Paris. Life with young children in the city was not always easy for Dottie, who had one underfoot at home and the chore of taking them all after school everyday to play for two hours in the *Jardin du Luxembourg*, overseen by elderly men and women who exercised draconian rules to prevent children from doing anything out of the ordinary, such as setting a foot on the grass. But the children adapted well to school and spoke good quality French and we all learned a lot about French life and good food and wine.

Our stay in France was a great step forward in my development as a microbial geneticist, due to a large extent to the interactions with people like Maurice Hofnung (we shared a lab), Hirota, Signer, Brenner, Monod, Eisen, and above all François Jacob. He has played an important role in my career and I have always been grateful for having such a great man as my *patron*!

MIDDLE YEARS IN THE MIDWEST

Resistance in Madison

Settling into life in Madison was not difficult, largely because we obtained a unit in University Houses next to a delightful couple with three children close in age

to ours, in an international community of families. After two years of living in an apartment in Paris, it seemed idyllic. Also we were familiar with the city from my postdoc years.

I was given adequate laboratory space in the Biochemistry building on the third floor next to Bob Wells, who had been in Khorana's laboratory, and quickly became a familiar face in the storeroom. I soon found a technician and "lab mother" as well as some students. The major problem was the topic of biochemistry, which I now had to teach, never having had any formal training in the subject. I was given responsibility for Biochemistry 201, a basic course for nurses, landscape architects, athletes, and anyone else who needed a survey of biochemistry without too much chemistry. To make up for my inadequacies, I designed the course to be taught with a group of graduate student teaching assistants, who held small group tutorials for discussion and problem solving. With an enrollment of 300 or more students, I needed lots of help. I found that good science course texts for nonscience majors were few and far between, so eventually I wrote *Elementary Biochemistry* with Barbara Littlewood, a senior postdoctoral fellow in the lab, to fill the need. Scientists from many countries have told me that they learned biochemistry from our book, which was translated into several languages, including Japanese.

In Biochemistry 201 I tried to give as many "everyday" examples of biochemistry as possible—cooking, detergents, wine and cheese-making, etc. One year, after describing the combination of denaturation and drying involved in making a meringue, Dottie and I worked all night to provide meringues for the entire class. We did it only once! This led to an offer of five bonus points on the final examination for student submissions of a dish that demonstrated some biochemical process. There were usually several dozen entries and the department faculty were invited to award the grades. Unfortunately, a group of female students thought that this was taking advantage of women, and despite the fact that male students also participated, we had to drop the scheme, which was certainly fun while it lasted and a useful exercise in applied biochemistry.

Once my laboratory was set up, I finished off a few *lac* crosses to round out a manuscript on the Paris work for the *Journal of Molecular Biology* (7) and went back to studies of antibiotic mode of action and resistance. It was now known that streptomycin resistance was due to an alteration in a ribosomal protein, and it seemed likely that the same would be true with other antibiotics. In addition, R-factors intrigued me because the resistance mechanisms were obviously different from those arising by mutation. My interactions with Hirota in Jacob's lab had convinced me that this topic merited investigation at the biochemical level. Takeshi Yamada, a postdoc from Japan, very quickly elucidated the mechanism of streptomycin resistance encoded by plasmid R100 (21). This was the beginning of a series of studies of plasmid-mediated aminoglycoside inactivation involving a number of students, postdocs, and visitors. A lot of the preliminary work on antibiotic modification in the *Streptomyces*-producing antibiotics was done with my first graduate student, Raoul Benveniste, a very skilled and productive experimentalist, and we published a paper on the possible origins of plasmid-determined resistance

Figure 5 With Masayasu Nomura—two small subunits at Cold Spring Harbor.

determinants in *PNAS* in 1973 (1). Patrice Courvalin spent two productive years at UW as a postdoc working on resistance mechanisms in gram-positive bacteria.

We also focused on protein synthesis inhibitors, and Terry Helser, another student, identified the nature of kasugamycin resistance in bacteria with the help of Jim Dahlberg (12). This interesting mechanism involving a change in a ribosomal RNA-methylating enzyme was a topic in the lab for many years. Our research benefited greatly from studies of the function, structure, and genetics of ribosomes by Masayasu Nomura (Figure 5), Bernie Weisblum, Gary Craven, and Chuck Kurland. There was a good nucleus of ribosomologists in Madison, and until the groups became too competitive, we collaborated successfully. From my viewpoint Masayasu's work was so good and comprehensive that it was pointless to try to compete with him.

Early on it seemed obvious that the antibiotic/resistance/mutation approach would work with eukaryote ribosomes, so I started studying the yeast *Saccharomyces cerevisiae*. Harlyn Halvorson had an active group in the Molecular Biology Laboratory working mostly on yeast mitochondria. Since at that time isolating mitochondrial ribosomes was very complicated, we decided to work with the cytoplasmic 80S ribosomes. Harlyn gave me a strain of *S. cerevisiae* and we immediately had troubles growing it. He failed to tell me that *S. cerevisiae* has to be grown at 30°C and we were using 37°C! Fortunately, Barb Littlewood, a trained yeast geneticist, joined our lab as a senior postdoc; she set us right and eventually we isolated a variety of antibiotic-resistant mutants and started genetic mapping and

biochemical studies on them. Soon the laboratory was actively studying mechanisms of antibiotic action and resistance in both bacteria and yeast, including chromosomal and episomal determinants.

At a Cold Spring Harbor Laboratories (CSHL) meeting I told Jim Watson about our budding work on yeast protein synthesis, and he invited me to spend the summer of 1969 in Cold Spring Harbor to do some yeast research there; once again I ventured into a field where I had no formal training. The brief stay at CSHL was very valuable. I attended all the courses, meeting and interacting with many of the best scientists in the field: John Cairns, Alfred Hershey, Barbara McClintock, Joe Sambrook, Ray Gjestland, Arnie Levine, Julie Marmur, Norton Zinder, Bob Webster, and always, Jim. (Happily, although there were threats of running me out of town when I asked for donations to fund a demonstration on the Bungtown Road in protest over the investiture of that upstart Charles as Prince of Wales, Jim and John managed to placate the local chief of police and I was allowed to stay.)

The Department of Biochemistry was a terrific place to work. The faculty was very strong and always attracted top students. There was a fine seminar program, a steady procession of visitors to our oasis in the Midwest, and excellent meetings like the Steenbock Symposia. With research groups such as the Enzyme Institute, Genetics, Molecular Biology, Zoology, and the McArdle Cancer Institute, UW was an enormously stimulating life sciences environment. Anytime one wanted to try a different kind of technique or do an experiment in another field, there was someone to help. This was vital to all our research, given the significant technical advances of the 1970s: restriction enzymes, recombinant DNA, electrophoresis, and then sequencing. Jim Dahlberg and Fred Blattner were the main support in sequencing, while Bernie Weisblum was the campus resource for restriction enzymes and cloning; he helped us many times (3). For many years he provided much of the EcoR1 enzyme used on campus. Having Bill Reznikoff and Bob Wells as lab partners on the third floor of Biochemistry made a huge difference as well; there was great interaction between our groups that extended beyond science. This was the “plasmid” era; the isolation and characterization of extrachromosomal elements from bacteria of clinical and environmental origin became a significant occupation in many laboratories, including mine. Plasmids were everywhere and were the first indication of the extent and importance of horizontal gene transfer. This research laid the groundwork for many of the techniques now employed in recombinant DNA technology. We had a brief flurry in discovering restriction endonucleases. David Smith, an undergraduate in the lab, screened our collection of clinical isolates (it seemed a good place to look) and isolated PstI (16), KpnI, and MboI in a week or so—it seemed too easy!

Transposition in Geneva

In 1974 I obtained an EMBO (European Molecular Biology Organization) Fellowship to work with Pierre Spahr at the University of Geneva. On the way I spent a productive month in London with Naomi Datta and her group at Hammersmith

Hospital. Naomi is an extremely gracious and generous person to work with; I taught them how to make restriction enzymes in exchange for a lot of useful plasmid information and strains.

I had no specific research plan for the sabbatical year in Geneva other than to work with antibiotic-resistance plasmids. Douglas Berg was a postdoc in Lucien Caro's lab, and we decided to collaborate in using bacteriophage λ to clone resistance genes out of bacteria. I gave Doug several strains carrying aminoglycoside-resistant plasmids through which he passed a bacteriophage λ mutant in order to isolate λ phage capable of transducing antibiotic resistance. The experiment worked surprisingly well, but in an unexpected manner: The resistance genes were transposable and had hopped onto the phage genome. Further studies with Bernard Allet and Jean-David Rochaix led to a demonstration of the structure of the transposon elements. Seeing stem-loop structures on electron microscopy of the denatured/renatured DNA was exciting—so much so that I learned how to examine DNA by electron microscopy to continue with this work in Madison. It was a successful sabbatical, with ski trips in the Alps, a week's holiday in the south of France, and publication of the isolation of Tn5 and Tn6 (both kanamycin-resistance transposons) (2). Doug Berg came to Madison and continued with this work while he sought an academic position in the United States. Bill Reznikoff also got involved, and the next few years were very transposon-oriented on the third floor with structural and mechanistic studies; we demonstrated many such elements on plasmids in both gram-negative and gram-positive bacteria.

We found earlier that the Tn5-encoded phosphotransferase expressed resistance to the broad-spectrum (and toxic!) aminoglycoside G418 (geneticin), and I thought that this might be useful for DNA manipulation in eukaryotic cells. When Antonio Jimenez came from Madrid for a sabbatical year, I suggested that he investigate this possibility, so he set about transforming *S. cerevisiae* with various Tn5-containing plasmids and testing for resistance to G418. To our great delight the Tn5-encoded phosphotransferase effectively protected yeast against G418 and we had ourselves a powerful selection for gene transfer in yeast (14). We looked for evidence of transposition, of course, but the results were ambiguous. Since the combination of neomycin phosphotransferase and G418 worked so well in a simple eukaryote, we tried similar experiments in mammalian cells but did not succeed, probably because of inexperience with cell cultures. Subsequently, I mentioned the experiments to Paul Berg (Stanford), and Peter Southern, a postdoc in his lab, succeeded in showing that the combination is a powerful selection system for all eukaryotic cells (17). This seemed worth patenting, but the Wisconsin Alumni Research Foundation thought "it would not be of any great use." In retrospect, I am pleased that we did not proceed, since this might have restricted its general use. The gene has been widely employed in plant research, and it was gratifying when, in 1989, French Anderson (a fellow postdoc at Harvard) and colleagues at NIH used the bacterial neomycin phosphotransferase gene for the first approved heterologous gene cloning in humans.

We dabbled a bit with fungal biology, in particular the analysis of a fungal ribotoxin, restrictocin, which Dan Schindler showed to be an extraordinarily specific ribonuclease capable of cleaving a single phosphodiester bond in 28S rRNA to inactivate the ribosomes and stop translation (15). We worked on this project intermittently in Paris and now carry on with the study in Vancouver. Having learned a lot about the protein toxin, we are trying to see if it can be used as a component of an immunotoxin.

The laboratory got more and more involved with streptomycetes; we looked for plasmids and thought (incorrectly) that we had found them. I wanted to develop some research on biosynthesis of antibiotics, so I arranged a summer in the "streptomycete mecca," David Hopwood's laboratory at the John Innes Institute in England. This was scientifically successful and very enjoyable; I became comfortable handling these beautiful bacteria and met many of the key people in the field. Working in David's lab convinced me of the importance of the combined genetic and molecular analyses of biosynthetic pathways, and being in Norwich when the first streptomycete cloning vectors became available got me off to a good start. (I also enjoyed playing for the John Innes squash club and pleased my father immensely by scoring 40-not-out for their cricket team!)

Our work on the mode of action of protein synthesis inhibitors and on mechanisms of antibiotic resistance led to many good associations with the pharmaceutical industry. I had consultancies with Upjohn, Bristol Myers, Eli Lilly, and Schering Plough that were valuable to my research in many respects. Although my first honorarium was spent on "the Upjohn dining table" around which family and friends still gather, most of the honoraria went to the lab or to the department for seminars or entertaining visitors. More importantly, the visits to company laboratories gave valuable insight into how research is done in the pharmaceutical industry, particularly with respect to antibiotic discovery and development. Although I was not always impressed with the science, which seemed to be constrained by torpid decision making and compartmentalization of the research, my contacts with company microbiologists, biochemists, chemists, and clinical specialists taught me a lot about antibiotics, and the lab profited from the many compounds and strains generously provided for academic research.

Unfortunately things have changed, and although successful academic/industrial interactions exist, they are far too often hampered by extensive bureaucracy and form-filling; this even extends to university/university interactions nowadays. Everyone is paranoid about losing something of potential value. The collections of strains and small molecules held by pharmaceutical companies are an enormous resource that is very poorly exploited; if academics had access to compounds for biochemical testing, I am convinced that useful drugs would be discovered and much interesting biology carried out. Natural products are diverse in structural and biological properties; the problem lies in knowing what type of activity to look for. Why not let more people look?

The 1970s at UW had many highlights, including Nobel Prize awards to Gobind Khorana and Howard Temin, both richly deserved. The period was also enhanced

by participation for a number of years on a study section for the National Institute of Allergy and Infectious Diseases (NIAID). It was hard work, but the Washington meetings were always lively and worthwhile, providing the opportunity to interact with many of the leaders in the field. Stan Falkow was one of these, and we had (and continue to have) a very positive symbiotic relationship.

Madison was a good place to live, even if the winters could be perishingly cold, the summers hot and humid, and the political climate volatile. School activities and various sports were enjoyed; I coached several soccer teams and even led a youth team on a tour of Europe, which was a good experience for them but harrowing for me! Many productive students, undergraduates and graduates, postdoctorals, and sabbatical visitors came to work in the lab. People such as Takeshi Yamada, Mori Yagisawa, Tom White, Eric Cundliffe, Mike Cannon, Patrice Courvalin, Wolfgang Piepersberg, Antonio Jimenez, Jan Westpheling, and Ken Komatsu made our laboratory truly international and a stimulating place to be. Altogether the UW-Madison years were exceptional—and I learned some biochemistry.

Why did I leave Madison? Perhaps the main reason was that biotechnology seemed an exciting opportunity to exploit basic science in new ways. When Wally Gilbert asked me to become scientific director of Biogen, then a small biotechnology company in Geneva, Switzerland, I was ready for another challenge. We had been in Madison for 13 years, and although I was very happy in the Biochemistry Department, I did not want to grow old there. I was concerned about leaving my students, but Biogen let me travel back to Madison on a regular basis to work with them, and two of my graduate students finished their Ph.D. work in Geneva; both went on to good careers with biotech firms.

AN UPSTART AT A START-UP

The company did everything possible to help us get settled, and from our house near the French border I could bicycle through the countryside to the lab. When we arrived, there were no more than a dozen employees, including the administration. My first task was to build a group of outstanding people doing good applied science, and the company soon had an excellent research team—with no grant applications! It was a scientific utopia (for a while). We were in the business of exploiting recombinant DNA technology for everything possible, looking for projects that would bring significant up-front payments to fund Biogen's development. Thus business rapidly became one of my roles and once again I had to learn as I went along. I benefited greatly from working with the company's CEO, Rob Cawthorne, who taught me a lot about business proposals and negotiating them. It was agreed that a scientist would always accompany a member of the business group at most stages of the interaction with a potential collaborator. Rob and I worked comfortably as a team and made several forays in other countries before he left Biogen to become the CEO of a U.S. pharmaceutical company. Over time a number of profitable agreements were signed and before long Biogen-Geneva

Figure 6 The Biogen Scientific Advisory Board in 1983.

had more than 200 employees from many different countries, in this sense the first multinational "biotech."

The Scientific Board was a powerful collection of fine scientists and I appreciated their intellect and frinedship—but not the efforts of some of them to run the labs (Figure 6). The science done in the Biogen laboratories on gene isolation, manipulation, heterologous expression, protein isolation, and purification was at the cutting edge, and I learned a lot at first hand about recombinant DNA technology and making proteins (such as growth factors, interleukins, and hemopoietic factors) on a large scale. The work was exciting and in retrospect enormously important to the development of immunology and cell biology, since it provided pure active proteins where previously only ill-defined reagents such as "conditioned media" had been available. This is a spin-off of the "biotech revolution" that is not appreciated. We also worked on recombinant DNA vaccines: hepatitis B vaccine was successful, but foot and mouth disease vaccine was not; this latter problem remains unsolved. Biogen and Meiji Seika Kaisha in Japan negotiated one project on small molecules, run by Charles Thompson, on the application of genetic engineering to the overproduction of the herbicide bialaphos. When the first recombinant DNA library in streptomycetes was made, everyone in the company participated in the difficult job of picking a few plates of colonies off agar to use in screening. The project was a success, but not quite in the way we had anticipated. Although Biogen generated high-producing clones, the most important result was the isolation and identification of the bialaphos-resistance gene, which was employed in the generation of bialaphos-resistant plants in collaboration with Marc van Montagu's group in Ghent and developed successfully for agricultural use (19).

In 1985 I made a difficult decision. Biogen had moved its base to Cambridge, Massachusetts, and was becoming a "big" company with an increasing emphasis on clinical development (which I did not always understand and thought premature) and we were getting weary of my constant traveling. Most significantly, no longer was it exciting to go into work each morning. The time seemed right to accept François Jacob's invitation to move to the new Biotechnology Department being organized at the Pasteur Institute. Our leave-taking from the Biogen family in December 1985 was a sad occasion; we had benefited greatly from the companionship of many good friends. Who could forget the ski trips and wine expeditions, or convivial discussions over lunch in the local bistro? We had done fine science together, contributing significantly to the development of recombinant DNA and its applications in the pharmaceutical industry. Obviously Genentech, Cetus, and Amgen (our major competitors, but also friends) were equally effective; each had its unique company culture, but all were based on the excitement of hypothesis-driven research and its applications. It would be impossible to reproduce the exhilaration and expectations of the emerging biotechnology industry.

PARIS ENCORE

The move to Paris was no more difficult than other transitions. Although the French customs officials were obstinate when I arrived at the border with the trunk of the car half-filled with bacterial strains and sheaves of official documentation provided by the Swiss side, sending my collection to Paris by train miraculously solved this problem. We found an apartment on *Boulevard du Montparnasse*, a comfortable walking distance from the Pasteur Institute in a *quartier* that we knew. I fortunately engaged an excellent administrative assistant, Helena Sinno, who spoke good English. (I myself spoke "fluent bad French," I was told.) The Biotechnology Department was in a new building with some construction still in progress, but Helena and I moved into our space on the fourth floor and got underway. Not surprisingly, an espresso machine had high priority. To my great pleasure Pascale Cossart, Brigitte Gicquel, and Philippe Mazodier indicated a desire to move to the newly formed *Unité de Génie Microbiologique* (UGM) and the research plans were set in place. I was interested in beginning some molecular genetic studies of bacterial pathogenicity, and happily this coincided with the objectives of Brigitte and Pascale, who set up groups working on *Mycobacterium tuberculosis* and *Listeria monocytogenes*. Philippe worked on streptomycetes and the *Unité* also did studies with filamentous fungi (the toxin). In 1987 Charles Thompson left Biogen to join UGM, making us a very active research group of more than 20 people involved in a variety of microbial projects, generating good publications. We were early into proteomics with the research that Charles and Tom Holt did on cell development in streptomycetes (13). My own interest in small molecules and their roles in microbial function (and possibly evolution) matured, and a review was published in 1990 (4). This led to a productive collaboration with Renée Schroeder (Vienna)

during which we studied many aspects of small molecule biology involving RNA reactions (20). Once again our lab became international in flavor with visitors from North America, Europe, and Japan.

One could not say that working at Pasteur was problem-free; the administrative and bureaucratic complications posed by the Institute and government organisms were often baroque. The frustrations of dealing with the administration were discouraging, with unforeseen problems a routine occurrence, and certain colleagues seemed unwilling to put up a fight. However, UGM succeeded more often than it failed and, thanks to the persistence of Pascale, Brigitte, and Charles, not to mention the efforts of Helena, gained good support and considerable research success. Certainly my years at Pasteur taught me how to deal with almost any type of bureaucracy!

There were numerous funding opportunities in the European Economic Community (EEC), and in order to maintain an active research program it was essential to take advantage of as many of these as possible. The EEC promoted collaborative efforts effectively, and our laboratory participated in a project on the examination of gene transfer between plants and genetically modified bacteria that involved labs in France, Germany, Spain, and Greece. Although there were certainly administrative problems with such joint initiatives (there was no common currency then and umpteen more layers of bureaucracy), the opportunities to interact with scientists in different countries led to scientific collaborations (not to mention the chance to get out of Paris and spend a few days in sunny Barcelona or Thessaloniki in winter). EMBO is a great example of international cooperation; it does an incredibly good job with workshops, courses, and training programs in all aspects of molecular biology. I have long wished that a North American organization could be funded by the U.S., Canadian, and Mexican governments to develop a similarly interactive program, but there seems to be little enthusiasm—perhaps the name NAMBO puts people off!

I was occasionally asked to aid in business matters at the Pasteur Institute and became a consultant for Rhone-Poulenc Santé and Roussel-UCLAF. Most enjoyable was a relationship with the company Moët & Chandon, investigating the role of the bubbles in champagne. The opportunity to learn, at first taste, about great champagne was not to be missed. In addition to giving me valuable experience in working with different types of business enterprises, these various associations provided extra funding for the Unité. I was also involved in establishing Xenova, a small start-up company in England focused on developing natural product therapeutics. With a Board made up of a diverse group of excellent scientists, the discussions were always spirited, although management often failed to implement our recommendations. I had the impression that my visits to Xenova were much anticipated, since I usually arrived from Paris with a few dozen fresh croissants from a local *boulangerie*.

Paris was a valuable and enjoyable part of my life, both personally and scientifically. Dottie and I went to the opera and concerts and had several favorites among the dozens of small restaurants in our area of Montparnasse. We sampled

the bread at most of the *boulangeries* between our apartment and Pasteur and soon learned where to find the baguette we liked best and whose croissants were flakiest. And of course, the occasional visit to a *grande table* was always an experience (gastronomically and financially). We had a small country house near the coast in Normandy that was a great source of pleasure to us: a way to refresh mind and body after the hustle and bustle of the city.

MOVING ON

In fact, the frenetic pace of daily life in Paris was part of the reason for our move to Vancouver. In 1991, when the Dean of Science of the University of British Columbia, at the suggestion of Mike Smith, telephoned to ask if I might be interested in the Headship of Microbiology and Immunology, we were willing to consider the possibility. The city of Paris is beautiful. I worked with a wonderful group of people at the Pasteur Institute and was very satisfied with the accomplishments of UGM, not least the maturing of Brigitte, Pascale, and Charles into leaders in their respective disciplines, the result of their ambition and hard work (with a little help from me). But Vancouver has its own beauty; some of our family live nearby; there was the promise that in Canada I could have lab space to continue with research for another 20(?) years —and might there be new adventures in business? We'll leave that for the next chapter, if ever I write it.

I cannot imagine working in anything other than the life sciences, especially small molecule biology, be it in academia or industry. Teaching and research are my passion, and this has probably been difficult for my family on occasion, especially during the children's school years. But none of them now expresses regret at having been exposed to other cultures or all those scientific types. For me it has been a privilege to be part of the scientific community and I am happy to serve in any way I can; election to the Presidency of the American Society for Microbiology was a great honor and an opportunity for me to pay my dues. As a member of the international network, I have benefited from the expertise, training, and friendship of many scientists. Chemists like Stork and Khorana and molecular biologists like Watson, Gilbert, Jacob, Brenner, Nomura, and Hopwood are my scientific heroes, and I thank them and all my colleagues over the years for teaching me how to appreciate science and in particular the wonderful world of microbes. Study of the unfathomable diversity and universality of these least known of living organisms contantly reveals novel and unexpected information on the evolution and function of life.

The students and postdocs who have come from a variety of countries and educational backgrounds to work in my labs have enriched my life with their curiosity and hard work; their ideas and speculative side projects have constantly nourished the ongoing work in the lab. Contributions such as theirs are essential to our science, and I take some satisfaction from their success in research, business, and law.

The practice of science has provided me with a lifetime of good experiences, lots of fun, and only minor disappointments. Admittedly there have been frustrations in academia (resources are rarely adequate) and anguish in business ventures ("downsizing" is traumatic for everyone), but overall the excitement of following lines of reasoning or leaps of imagination to a new finding is more than enough compensation. For me scientific discovery is the greatest thrill of all—except perhaps playing rugby for Wales. If the Welsh Rugby Union asked me, I'd be prepared to make another move. After all, a rolling stone may gather no moss, but who wants to be covered with that stuff?

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