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Neutrons, Magnets, and Photons: A Career in Structural Biology

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The purpose of Reflections articles, it seems, is to give elderly scientists a chance to write about the "good old days," when everyone walked to school in the snow. They enjoy this activity so much that your editor, Martha Fedor, must have known that I would accept her invitation to write such an article, no matter how much I demurred at first. As everyone knows, flattery will get you everywhere. It may comfort the apprehensive reader to learn that there is not going to be much walking to school in the snow in this story. On the contrary, rather than thinking how hard I had it during my scientific career, I find it inconceivable that anyone could have had a smoother ride. At the time I began my career, science was an expanding enterprise in the United States that welcomed the young. Only in such an opportunity-rich environment would someone like me have stood a chance. The contrast between that world and the dog-eat-dog world young scientists confront today is stark.

Beginnings

I was born October 15, 1939, about six weeks after Hitler invaded Poland, a bad day in a very bad year. The fact that I was born in Boston instead of Warsaw, for example, saved me from the fate suffered between 1939 and 1945 by all too many of the others born that year, but as far as the rest of my life was concerned, the timing of my arrival could not have been improved upon.

In the era that I went through elementary school, teaching was one of the few occupations fully open to women, and consequently, to the incalculable benefit of generations of grade school children, including my own, it attracted more than its fair share of capable women. My high school teachers were just as remarkable. My parents sent me to a small private school outside Boston called Milton Academy. Most of my teachers were veterans, who had done a lot of living between 1941 and 1945, and they did not take guff from adolescents, which was good for all concerned. Two of them had a particularly large impact on my subsequent life: Donald Duncan, who taught me calculus, and Harry Stubbs, who had worked some graduate training in astronomy into a career that included flying in B-24s in Europe. Among other things, he taught an elective course in astronomy that met in the evening so that its students could use a small observatory on the school grounds. It was my first encounter with real science, and I loved it; I might have become an astronomer.

For centuries prior to 1957, the year I left Milton, all the graduates of Boston area prep schools who could fog a mirror had attended Harvard College, and although the strength of that tradition was waning by that time, that is what many of my classmates did. I went to Yale University instead. When, in some random encounter that spring, my mother informed an elderly Bostonian of this startling turn of events, there was a brief pause while the matron struggled to find something polite to say, and then the remark, "Oh, how original!"

By the time I finished my sophomore year in college, I knew that I did not want to follow my father into medicine, but that I was intrigued by the chemical/physical aspects of biology. Yale was one of the few colleges that had a biophysics department in those days, and because its undergraduate program was well aligned with my interests, I signed on. By the fall of my senior year, I had decided to obtain a Ph.D. in biophysics.

Graduate School

Scientists of my generation owe a lot to the Cold War. If there had been no Cold War, the citizens of the United States would not have felt the consternation they did when the Soviet Union put Sputnik into orbit in 1957, and Congress would not have authorized the massive increases in the funding for science and technology that ensued. So it says more about the times than it says about me that every grant application that I submitted in the first decades of my career got funded. For example, I obtained a graduate fellowship from National Science Foundation (NSF) to pay for my graduate education. (I note in passing that, in those days, fellowship income was tax-exempt; they really loved us.)

In the spring of 1961, I had to decide where to use my fellowship, and after some agonizing, I accepted the offer I had received from Harvard's Committee on Higher Degrees in Biophysics, which turned out to have been a good decision. I had no idea what kind of research I wanted to do at the time, and students in that predicament, which most beginning graduate students either are or should be, are better off in large programs like Harvard's than in small ones because the range of choices they offer their students is wider.

In those days, Ph.D. candidates were required to demonstrate their ability to read two foreign languages, which is all I will say about walking to school in the snow, and students in Harvard's biophysics program were expected to take four courses a term for two full years. As everyone knows, as far as science Ph.D. programs are concerned, language requirements have gone the way of the hoop skirt, and no graduate program I know of requires anywhere near that much course work. Everyone is entitled to his/her own opinion about the wisdom of allowing our students to go through life ignorant of foreign languages, but there can be no doubt that, by cutting formal course requirements, we have acquiesced in a reduction of both the depth and the breadth of our students' educations. In the long run, that cannot be a Good Thing for them or for science.

I spent the summer of 1962 doing research on chloroplast development in a botany laboratory, concluded that botany was not for me, and that fall asked James (Jim) Watson for a place in his group, which he granted me. Although I did not appreciate it at the time, I now know that a graduate student's choice of a thesis advisor is likely to be the most momentous professional decision he or she ever makes. Nothing that has happened to me since would have occurred had I worked for someone else.

In the 1960s, Jim ran his group in collaboration with Walter (Wally) Gilbert, who divided his time between teaching quantum electrodynamics in the Physics Department and doing molecular biology in the Biology Department, which was an unusual parlay, to put it mildly. Their group was a wonderful place for students, not least because of the extraordinary quality of the students and postdoctoral fellows it attracted.

Jim put me to work studying the interaction of messenger RNAs with ribosomes and ribosomal RNAs. It turns out that mRNAs bind to rRNA under the same ionic conditions that they bind to ribosomes, and Jim must have hoped I would demonstrate that the mRNA-binding activity of the ribosome derives from rRNA. We now know that it does, but my data did not support that hypothesis. Not only does naked 23 S rRNA bind mRNA at least as effectively as 16 S rRNA, which it should not have because mRNA binding is specific to the small subunit, but also the primitive chemical modification experiments I did indicated that the rRNA/mRNA binding results from conventional base pairing but that ribosome/mRNA binding does not (1, 2).

In that era, all graduate students had to do to get their degrees was to demonstrate that they could contribute to their chosen fields; they did not have to publish first-author papers in *Nature* or *Science*. Jim thought I had crossed that threshold by the end of the summer of 1965, and in September, he told me to start writing. In those days, you were supposed to write your thesis in no more than a month or two; students were actively discouraged from writing the scientific equivalent of *War and Peace*. I submitted my thesis on November 9, 1965, a date that will live forever in memory not because of my thesis, I regret to say, but rather because of the power failure that engulfed much of the northeastern United States and parts of Canada that evening.

Like many scientists of his generation, Jim long felt that women should not pursue careers in science, and consequently, it was not until 1964 that he accepted his first female graduate student (Joan Argetsinger, now Steitz). On the other hand, Jim was happy to have young women

in his laboratory. Radcliffe undergraduates were highly acceptable, and his group invariably included a number of slightly older female technicians. Their presence brightened the lives of the male members of the Watson group and afforded them opportunities of a nonscientific sort, of which advantage was taken by many. During my last term at Harvard, I managed to convince Margaret Murphy, a recent graduate of Northwestern, that marrying me would be more interesting than continuing to help Wally Gilbert pursue his seemingly forlorn search for the *lac* repressor.

Postdoctoral Days: Geneva

About a year before I finished up, I had a chat with Jim about what might come next, and he told me that Alfred Tissières, who had worked with Jim at Harvard before my time and whom I knew by reputation, was setting up a new laboratory at the University of Geneva. Because ribosomes were on Alfred's agenda, and there were things I wanted to do with ribosomes, I decided that it would be a good idea to go to Geneva as a postdoctoral fellow.

So it was that, at the beginning of February 1966, I headed off to Switzerland, equipped with a new wife and a new NSF fellowship. Shortly after my arrival, I had a discussion with Alfred about research, in which I told him that I wanted to purify and characterize the ribosomal proteins from *Escherichia coli*. He greeted this proposal with his usual enigmatic smile and suggested that I might be wise to collaborate with Robert (Rob) Traut, another American postdoctoral fellow, who was already working on the same problem.

In the early 1960s, while working for John Edsall at Harvard, Jean-Pierre Waller and Ieuan Harris had discovered that methionine is the dominant N-terminal amino acid in ribosomal protein isolated from *E. coli*. Because no one then understood how protein synthesis is initiated, that observation was interpreted as indicating that the number of different proteins associated with the ribosome might be small (3). However, a series of protein fractionation and starch gel electrophoresis experiments Waller had done on ribosomal protein at the same time suggested that ribosomal protein might be a mixture of >20 different components (4), a conclusion resoundingly supported by acrylamide gel electrophoresis experiments done subsequently (5). Adding to the confusion, there were rumors that tryptic digests of ribosomal protein contain only ~20 peptides, and if true, that observation would have strongly supported the conclusion suggested by end group studies. The only way to settle the matter was to purify and characterize the components in bulk ribosomal protein, however many there might be, and that is what we set out to do.

Rob and I agreed that he would continue doing the electrophoresis and fingerprinting experiments he had already embarked upon while I tried to find a way to fractionate bulk ribosomal protein. I had no background whatsoever in protein chemistry, and it took me several months of flailing around with that poorly soluble glop to appreciate just how far Waller had gotten with it. I began repeating his experiments, one of which involved fractionating ribosomal protein by chromatography on carboxymethylcellulose (CMC) columns in urea-containing buffers. The only thing I did with my CMC columns that Waller had not done with his was to elute them with salt gradients instead of stepping proteins off them batchwise. From the moment Rob ran samples from my first CMC column on gels, we knew we had a purification method that would yield milligram quantities of proteins that ran as single bands on acrylamide gels. At about that juncture, Harry Noller joined the Tissières group as a postdoctoral fellow. Some readers may be surprised to learn that Harry was then a *bona fide*, card-carrying protein chemist, which was just what we needed, and as it turns out, Harry came to Geneva from the Medical Research Council (MRC) Laboratory of Molecular Biology in Cambridge, where he had worked with Ieuan Harris; it is a small world. As amino acid compositions and tryptic fingerprints began to accumulate, all doubt about the complexity of ribosomal protein mixtures vanished (6). They are complicated.

By the spring of 1967, I was measuring the molecular weights of pure ribosomal proteins and trying to determine their stoichiometries in the ribosome. By then we knew we were not alone. Alex Bollen had made a lot of progress fractionating ribosomal proteins in Brussels. Charles Kurland, Gary Craven, and Masayasu Nomura were advancing rapidly in Madison, Wisconsin, and Heinz-Günter Wittmann was hard at it in Berlin. The first papers from Wittmann's group had come down on the simple side of the simple/complex controversy, but he was quickly persuaded otherwise, and his group went on to become a major force in the ribosome field.

Both we and the groups in Madison measured the molecular weights of ribosomal proteins by sedimentation equilibrium, which was a tricky undertaking because the buffers used had to contain high concentrations of urea to suppress aggregation. Measurements of this sort demonstrated that the average molecular weights of unfractionated 30 S and 50 S proteins are both ~15 kDa. Our gels suggested that the 30 S subunit contains ~20 proteins and that the 50 S subunit contains ~30, consistent with our CMC elution profiles. By multiplying both numbers by 15 kDa, you could estimate what the protein contents of the

two subunits would be if they were both 1:1 complexes of all their components, and those estimates corresponded well to their actual protein contents. Thus, it appeared likely that the ribosome is a 1:1 complex of all of its macromolecular components. It was much harder to prove this point in detail, one protein at a time, not least because of the technical difficulty of estimating the relative amounts of each protein in the ribosome. We also got into trouble because some of our molecular weight estimates were much lower than they should have been for reasons I still do not understand (7).

Our conclusion that the ribosome is a 1:1 complex was instantly challenged by Kurland and Craven, most of whose protein molecular weight estimates, it turns out, were somewhat higher than they should have been (8). Their data suggested that there is less than one copy per ribosome of many ribosomal proteins. It turned out, in the end, that the 1:1 conclusion is basically correct but that there are important caveats (9). First, one protein, which is called L7/L12 in bacteria, is found in multiple copies in all ribosomes. Second, another protein, S1, which is by far the largest ribosomal protein in *E. coli*, is always present in substoichiometric amounts. Third, the washing procedures commonly used to remove contaminating proteins and tRNAs from ribosomes invariably strip ribosomal proteins from them. A large fraction of the experiments that have ever been done to elucidate the mechanism of protein synthesis were carried out using ribosome preparations that had been damaged this way, an embarrassing fact that has received little notice over the years.

Postdoctoral Days: Cambridge

By the time the stoichiometry controversy came to a boil, I had left Geneva for what turned out to be a two-year sabbatical from the ribosome. Jim had intervened again. During a visit to Geneva in 1966 or 1967, he had suggested that I would be unwise to come home before spending some time at the MRC in Cambridge. He also told me that Hugh Huxley might have a position open, and so the requisite letters were exchanged. Even though I knew nothing about muscle, which is what Hugh worked on, he agreed to take me on, and the United States Air Force Office of Scientific Research provided a lush fellowship (about $10,000) to finance the venture. In the fall of 1967, Margaret and I packed everything we owned into a VW station wagon, which led to some rather hostile questioning from French customs officials when we crossed the Swiss border into France, and drove off to Cambridge.

Hugh's group was a component of what was then the most interesting biological research institution on the planet, which it may still be today. The place was packed with bright postdoctoral fellows and students, and the permanent staff was off-scale. Francis Crick, Max Perutz, Sidney Brenner, Huxley, Aaron Klug, and Fred Sanger were all in regular attendance, and John Kendrew could be spotted from time to time. "Packed" is the right word to describe the occupancy of the place. The population density was so high you had to fight for every foot of bench space; it is one the (many) reasons the MRC was so productive in that era.

As luck would have it, I was given a desk in the same office as David DeRosier, another American postdoctoral fellow. David was working with Klug on reconstructing the three-dimensional structure of the tail of bacteriophage T4 from (two-dimensional) EM images (10). The technique they had devised could be applied to the EM images of any macromolecular assembly that has helical symmetry, and muscle is full of such things. Before long, I was helping David write computer programs for doing helical reconstructions, taking electron micrographs to obtain the images needed for reconstructions, and mining Hugh's incomparable collection of muscle-related micrographs in hopes of finding images better than the ones I was producing, which were not hard to find. Two papers emerged from this work, one reporting the first EM reconstructions of actin and decorated actin filaments and the second dealing with the technical details of helical reconstruction (11, 12).

I have fond memories of an incident that occurred shortly after we had written a program that would make a line printer produce representations of matrices that resembled half-tone images. One morning, after we had tacked an image of a Fourier transform to the bulletin board across from our office so we could look at it from a distance, Crick came along and asked David what it was. David replied, "That's reciprocal space. It is the only space we have around here."

From the point of view of what I did later, the two most important legacies of my Cambridge years were my discovery that I liked doing structural biology more than I liked doing molecular biology and the understanding I gained of how reciprocal space methods can be used for structure determination.

The Move to Yale

My postdoctoral years were the best years of my career, and I know that most of my contemporaries feel the same way. During those three years, I got to practice my trade at two outstanding institutions, surrounded by wonderful colleagues and unburdened either by concerns about funding or by responsibility for the welfare of others. Add

to that the privilege of living in Europe with sufficient economic means to enjoy its cultural riches, and you have something magical. (I have always been amazed at how few of my own students were interested in spending their postdoctoral years on the far side of the Atlantic.)

All good things must come to an end, and in the spring of 1968, I returned home to look for a job. There were jobs open everywhere because both science and higher education were expanding rapidly all over the country. The Yale Biophysics Department was one of several I visited. I did so not because I thought I was likely to accept an offer from Yale, were one to materialize, but rather to pay my respects to the faculty I had known as an undergraduate. However, things had taken a turn for the better in New Haven. The appointment of several new faculty members had revived a department that had seemed moribund in 1961. Furthermore, a conversation I had with one of those new people, Harold (Hal) Wyckoff, revealed that there was at least one person at Yale who thought about structure the way I did, and in that era, such people were rare in the United States. Shortly thereafter, I went to Boston to see my parents, and, while there, I talked with Jim. He agreed with me that Yale was the best of the options open to me, and that was the end of it because I already had an offer in hand.

That fall, I submitted duplicate grant applications to the National Institutes of Health (NIH) and NSF to support some ribosome biochemistry I thought might be interesting. In those days, both agencies routinely funded novices, and both agreed to support me. I accepted the grant from NIH because it provided more funds. (Some things never change!) Thus, I had an R01 grant in hand *before* I got to New Haven. Can you imagine that happening to a beginning assistant professor today? (I hasten to add that, in those days, by way of compensation, institutions did not give beginning faculty large startup packages.)

Margaret and I arrived in New Haven early in April 1969. We had left an England where the flowers were in bloom and the leaves green, and we were greeted by a New England landscape still gripped by winter. It took some adjusting to get used to that environmental change. It also took a while to get used to the idea that, as a newly minted faculty member, I was going to have to take not only intellectual responsibility but also financial responsibility for everything I did thereafter.

By the time we got to New Haven, the Biophysics Department had undergone another transformation. It had been absorbed into a new entity called the Department of Molecular Biophysics and Biochemistry (MB&B), which had been created by merging it with the Medical School's Biochemistry Department. It had faculty and teaching responsibilities in both the College and the Medical School, which are about a mile apart. Its new chairman, Frederick (Fred) Richards, was charged with making this hands-across-the-campus venture work. A lesser mortal might have failed.

As part of the merger deal, Yale gave Fred a large number of junior faculty positions to fill. I was deemed to have filled one of those positions, and about a year after I got to New Haven, Donald Engelman was hired to fill another. I do not remember the order in which the other young scientists Fred lured to MB&B succumbed to his blandishments, but the people in question were Joan and Tom Steitz (whom I knew both from Harvard and the MRC), David Ward, and John Cronan. Luckily, we all got along very well, and our interactions with each other and with the younger faculty who were joint appointees in MB&B helped make the 1970s very enjoyable socially, as well as unusually productive scientifically.

Neutron Days

Although my NIH grant had to do with ribosome biochemistry, I still harbored ambitions to continue working in the three-dimensional reconstruction field. For that reason, shortly after arriving in New Haven, I cobbled together a Fortran program that would enable me to compute the Fourier transform of an image and submitted the corresponding stack of IBM cards to the only computer available to me on campus, an IBM 360/50 that had nowhere near the capabilities of the cheapest laptop you can buy today. Its primary function was doing tasks for the university's accountants, which it did at night, and in the daytime, faculty were allowed to use it. Miraculously, my job ran the first time I submitted it, but when I looked at the output, I discovered to my horror that this trivial exercise had cost me $50!

In that era, if you were an impecunious faulty member interested in computing, Yale would absorb the cost, but if you had money, you paid, and I had money. It was communism, pure and simple, and at $50 per Fourier transform, I knew I would go broke before I got anything done. (To put $50 into context, my first NIH grant generously gave me about $23,000 a year in direct costs, exclusive of the few thousand it provided for equipment, and most of that was going to have to be used for personnel.) So I turned to the ribosome biochemistry that I had promised NIH that I would do, which looked to be a lot cheaper.

The projects we started pursuing had to do with the cross-linking of factors to ribosomes, with the role of ribosomal cysteine residues in protein synthesis, and with the functional and structural properties of ribosomal protein

S1. Several students and postdoctoral fellows worked on these problems in the 1970s: Margaret Schenkman, Seetharama Acharya, Michael Laughrea, and Nandini Ghosh. However, not long after that work started, a new venture came into view.

In the fall term of 1971, Benno Schoenborn, who was then a member of the Biology Department at the Brookhaven National Laboratory (BNL), gave an MB&B seminar in which he described all the wonderful things you might learn about biological structures by neutron scattering/diffraction. A month or two later, Don Engelman and I had a lunchtime conversation in which the idea emerged that if you were to label a pair of ribosomal proteins with deuterium, the resulting alterations in the neutron scattering profile of a solution of otherwise unlabeled ribosome might reveal the distance between them. That afternoon, I set about deriving an equation for the interference signal that would be observed if the two labeled proteins were spheres and discovered to my delight not only that there is such an equation but that the signal would, indeed, report directly on center-to-center distances (13). Shortly thereafter, I learned that Debye had published the same equation in the 1920s, but better late than never, I always say.

We got in touch with Benno, and through him and with his enthusiastic support, we were given access to the beamlines he was using at the High Flux Beam Reactor (HFBR) at the BNL. Our first trip to the HFBR took place in 1972, and if memory serves, my last trip occurred in 1990, not long before the Department of Energy shut it down for good for political reasons. The primary objective during that entire period was to determine the placement of the proteins in the 30 S subunit of the *E. coli* ribosome by triangulation using interprotein distances measured by neutron scattering.

The reason you could even think of doing experiments of this type was that in 1968 Peter Traub and Masayasu Nomura (University of Wisconsin-Madison) had discovered that the small ribosomal subunit can be reconstituted from its constituent proteins and RNA (14). I knew how to purify ribosomal proteins, of course, and after some fiddling around, I learned how to reconstitute 30 S subunits. Nevertheless, it took us several years to prepare for our first distance measurements. Through it all, Betty Freeborn, the technician I had hired in the summer of 1969 and who helped keep my laboratory running for about thirty years, was a tower of strength.

By the time we were ready to do the first distance measurements, we had done enough neutron scattering measurements on ribosomes to know that the signal-to-noise ratios of the signals we had to measure would be very poor. I can remember having trouble sleeping at nights because of my worries that our experiment might fail due to our inability to collect data that were accurate enough. We were saved from that fate by a remarkable area detector for neutrons that was built at the BNL by Veljko Radeka in collaboration with Benno. (In the interest of brevity, I will not describe all the other important things Benno did to make our experiments possible.) The first experiments worked as anticipated (15).

It took twelve more years to finish the job, and it would never have happened without the help of a succession of graduate students and postdoctoral fellows who did most of the heavy lifting. In the order in which they joined the enterprise, they were Jerry Langer, Dan Schindler, the now notorious Venki Ramakrishnan, I-Yu Sillers, Malcolm Capel, and Morten Kjeldgaard. Sadato Yabuki, a visiting professor from Japan, also contributed. X-ray and neutron scattering experiments of a different design that were done on the ribosome by Kevin Kearney and David Harrison also added to our understanding of its organization.

In addition to making pairwise measurements, we had to develop the theory for analyzing data of that sort and for constructing three-dimensional maps from the distance information the data provided. We also had to write all the software required. The latter would have been an expensive nightmare if I had not become a member of a faculty consortium called the WERMS. Its members were Hal Wyckoff, Don Engelman, Fred Richards, me (P. B. M.), and Tom Steitz (Fig. 1). Around 1975, all of the crystallography and x-ray scattering equipment owned by the WERMS was assembled in a common laboratory, and a program project grant was secured from NIH to pay for the science that was to be done there. Among other things, that grant enabled us to buy a DEC computer powerful enough so that none of us ever used the Yale Computer Center again.

Around 1986, the Howard Hughes Medical Institute (HHMI) decided to invest in the structural biology effort at Yale, and the WERMS group changed into the Center for Structural Biology (CSB), which included several new, HHMI-supported faculty: Paul Sigler, Axel Brunger, Bob Fox, and later Jennifer Doudna. In 1986–1987, a VAX 8800 computer was purchased using HHMI funds, and I still remember a meeting the WERMS had with the head of academic computing at Yale at that time, in which he asked us to agree to add our VAX to the cluster of computers he controlled. An intemperate exchange of views ensued, and it is a measure of Fred's very considerable

FIGURE 1. **WERMS circa 2000.** From left to right: Harold W. Wyckoff, Donald M. Engelman, Frederick M. Richards, Thomas A. Stetiz, and P. B. M.

management skills that the head of computing emerged from that meeting with all his body parts still attached.

It takes at least 74 pairwise distance measurements to locate 21 proteins in space, and there are 210 different pairwise distance measurements possible in an array of that size. We declared victory in 1987 when we could produce a 21-protein map based on the distances between 93 different pairs of proteins (16, 17). Fig. 2 is a depiction of that neutron map, which is sometimes referred to by the irreverent as the "balls model."

I revisited that model in 2001, shortly after the first atomic resolution structures appeared for the 30 S subunit (18). I am happy to report that the correlation between the distances we measured by neutron scattering and those revealed by the crystal structure is very high, but there are some outliers that probably represent biochemical errors of various sorts. However, the real problem with the neutron map is not the few proteins that are completely out of place because of gross errors in the data but rather the larger number of what might be described as local inversions in the structure. Had we known how to do it, and I still do not, we should have computed not just the map that corresponded best to the data, which is the one shown in Fig. 1, but also the half-dozen or so maps that fit the data almost as well. It is likely that one of the almost-as-good maps corresponds to the real structure better than the one we published, and it is certain that comparisons between those low residual maps would have revealed the local

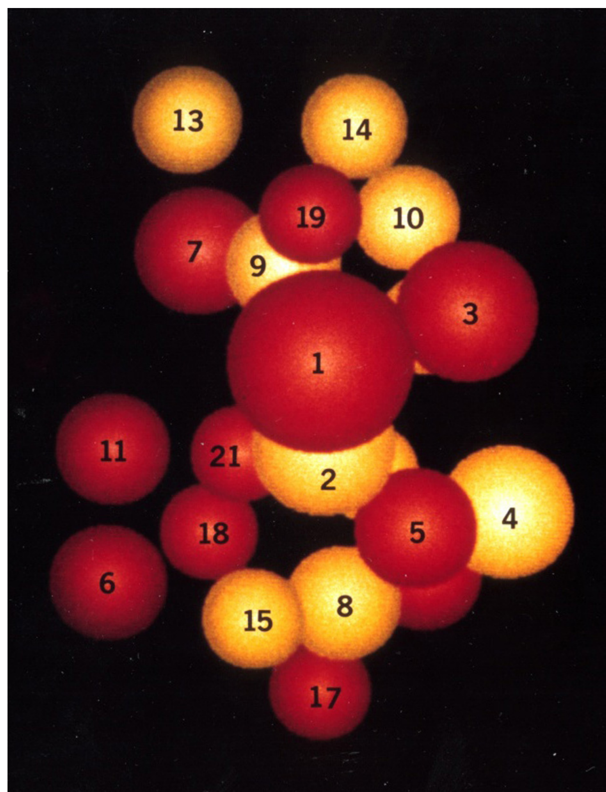

FIGURE 2. **Neutron map of the 30 S ribosomal subunit.** The model shown displays the positions that were found for the 21 proteins of the small ribosomal subunit from *E. coli* by neutron scattering (16). Proteins are identified using standard numbering. They are depicted as spheres that occupy the volumes a protein of that molecular weight would occupy drawn to the scale of the illustration. The model is viewed from its cytoplasmic side.

inversions I speak of, even if they would not have told us how to resolve them.

The reader may well be wondering why anyone would have made the investment we did in determining the structure of the small ribosomal subunit at such a crude level. It is important to remember that, at the time we started, there were no ribosome crystals, and, although the possibility of doing EM reconstructions on asymmetric particles had been discussed at the MRC in the late 1960s, it was decades before anyone seriously believed that the structure of the ribosome could ever be reconstructed at a resolution high enough to make a difference. Thus, between 1970 and 2000, all kinds of experiments were done to elucidate the structure of the ribosome in laboratories all over the world, most of them at least as crude as ours. For all anyone knew, the information obtained using that heterogeneous zoo of techniques would provide all anyone ever knew about the three-dimensional organization of the ribosome.

RNA and NMR

Around 1973, Yale entered a period of financial austerity from which it did not emerge for about two decades. Overnight, MB&B lost all the faculty slots it had been promised that were still unfilled, of which there were many, and it was not obvious that it would be allowed to retain any of its junior faculty. Nevertheless, somehow, almost all of us were "saved" in the end. David Ward and I were "rescued" by finding tenured positions for us in other departments, and so I moved to Chemistry in 1976, where I happily spent the rest of my career.

By the late 1970s, I knew that I did not want to scatter neutrons for the rest of my life, even though I am certain that NIH would have funded me to do so until the crack of doom; I had a franchise. My sense that I would be wise to look for alternatives was reinforced in 1979–1980, when I discovered that Ada Yonath and Heinz-Günter Wittmann had crystallized ribosomes in Berlin. Given what I thought we knew about the heterogeneity of ribosome preparations, their success came as a surprise. Nevertheless, even though it was obvious that atomic resolution structures could not be obtained from crystals that diffracted as poorly as those first ribosome crystals, their existence was warning enough that the days of the catch-as-catch-can, low resolution structure determination in the ribosome field were numbered.

Because the work my colleagues in Chemistry were then doing on macromolecules with NMR looked intriguing, I thought that it might be interesting to spend the sabbatical year I had coming up in 1979–1980 at Oxford with R. J. P. Williams. R. J. P. had access to one of the first 500-MHz NMR spectrometers in the world, and the idea was that I would learn about NMR by doing NMR experiments on some ribosomal protein samples I brought from home. I managed to sell this proposition to the Guggenheim Foundation. Margaret, our two young children, and I had a great time in Oxford that year, and I did what I had promised the Guggenheim people I would, more or less.

Shortly after returning to Yale, I got interested in an RNA called "fragment 1" that Roger Garrett (University of Copenhagen) had shown can be prepared from 5 S rRNA by limited treatment with RNase A. Fragment 1 forms a well behaved complex of workable molecular weight with ribosomal protein L25. Harry Noller gave me a strain of *E. coli* that overproduces 5 S rRNA, and using it, we could easily make 100-mg quantities of fragment 1. About that time, Matthew Kime joined the group. Matthew had been a graduate student with R. J. P. and had worked with me at Oxford in 1979–1980. He began doing imino proton spectroscopy on fragment 1 and its L25 complex using a 490-MHz spectrometer that had recently been purchased by Yale using funds obtained from NIH by a group of faculty led by James Prestegard. The spectra were beautiful; our first fragment 1 NMR paper appeared in 1983 (19).

The technology for performing macromolecular NMR evolved very rapidly in the years that followed. Huge improvements in instrumentation coupled with major conceptual advances led to the development of powerful new experimental techniques that dramatically enhanced both the quality and the quantity of the information that could be obtained about macromolecules by NMR. In the early 1980s, when we started, it was a challenge to assign the downfield spectra of RNAs, and twenty years later, people were routinely obtaining solution structures for 50-nucleotide RNAs. (The progress in the protein NMR field was even more dramatic.) The reason that NMR attracted as much attention as it did in those years from the RNA fraternity was that, from the mid-1970s, when the first tRNA crystal structures appeared, until 2000, when the first ribosome crystal structures were published, *very* little was learned about RNA structure by crystallography. NMR offered a credible means for combating that drought, even though NMR is better suited for studying proteins than it is for investigating RNAs.

It turned out to be much easier to get students to work on projects that depend on NMR than to persuade them to do neutron scattering experiments. Consequently, once Matthew Kime got the ball rolling, I was never short of students and postdoctoral fellows interested in doing NMR projects, and in the end, we obtained structures for a

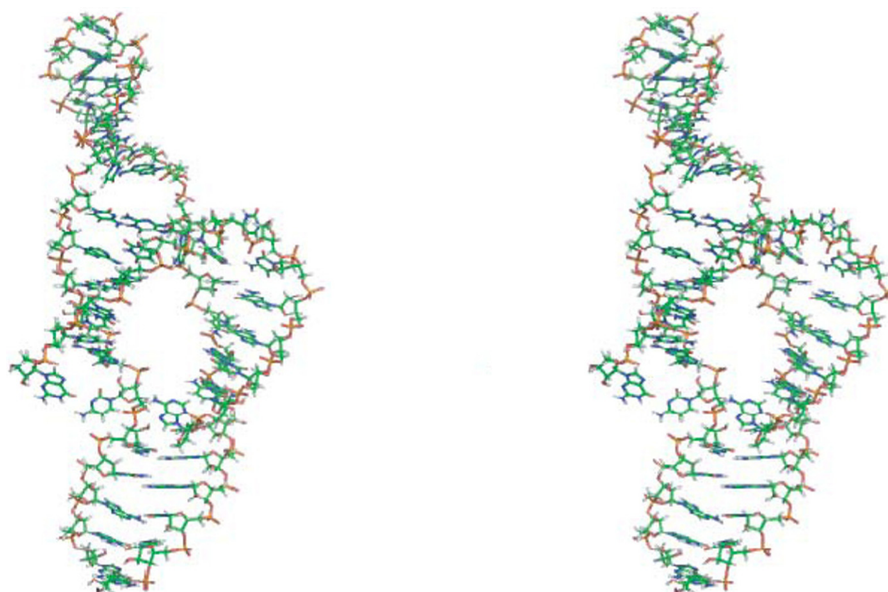

FIGURE 3. **Stereo view of the solution structure of a fragment of human 28 S rRNA bound to the central loop of the upstream stem-loop of human U65 small nucleolar RNA (snoRNA).** This NMR structure, which is the last of a long series of such structures produced by members of my group, was generated by Dr. Hong Jin (48). U65 snoRNA is a box H/ACA snoRNA that is involved in the pseudouridylation of rRNAs.

lot of interesting macromolecules, most of them RNAs (*e.g.* Fig. 3). The people who did the work were (approximately in the order they joined the group) Dan Gewirth, Steve Abo, Neocles Leontis, Partho Gosh, Penghua Zhang, Paul Popieniek, Bob Rycyna, Alex Szewczak, Susan White, Joe Kim, Joon Cheong, Greg Kellogg, David Schweisguth, Ann Dallas, John Diener, Sarah Stallings, Kathy Seggerson, Jason Rife, Paul Huber (who was a faculty visitor from Notre Dame), Marieke Bloemink, Josh Warren, Phramodh Vallurupalli, Cathy Turner, and Hong Jin.

Crystallography

NMR spectroscopists make a lot of noise about their ability to address the structures of macromolecules that cannot be crystallized. However, few of them will admit in public that the same physical properties that make a macromolecule a good target for NMR, *e.g.* solubility at concentrations above 5 mg/ml combined with a low tendency to aggregate, also make it suitable for crystallization. I first became aware of this fact in 1983 as a result of a lunchtime conversation with a postdoctoral fellow in Tom Steitz's group, Sherin Abdel-Meguid. After he had listened to me carry on about the wonders of fragment 1, he asked if he could have some for crystallization trials. Within days, he had crystallized it, and shortly thereafter, he obtained crystals of the L25-fragment 1 complex (20). His success marked the beginning of what turned out to be a long series of crystallographic projects that my group carried out in collaboration with Tom's group. Fragment 1 turned out to be a tough problem. It was not brought to bay until 1997 (!) (21), and it took a few more years for Tom's group to bag the L25 complex (22). The sarcin-ricin loop of 23 S rRNA, which we characterized spectroscopically in the early 1990s in collaboration with Ira Wool (University of Chicago), provides another case in point (23). It also was a "good" RNA spectroscopically, and its structure was later solved crystallographically in Tom's group (24).

In the intact ribosome, the sarcin-ricin loop interacts with all the protein synthesis factors that are GTPases, and Ira had evidence that it binds in isolation to elongation factor G (EF-G). These observations led us to look into obtaining a structure for EF-G crystallographically. Thus, about the same time our NMR studies on the sarcin-ricin loop were coming to fruition, I persuaded John Czworkowski to work on EF-G. I do not remember how we came to concentrate on the EF-G from *Thermus thermophilus* as opposed to, say, the EF-G from *E. coli*, but that is what we did. John got crystals quite easily, but they were horribly unstable. They would shatter within seconds after the chamber in which they had grown was opened to the atmosphere. This problem was solved by (carefully) cross-linking them with glutaraldehyde, as Greg Petsko (Brandeis University) recommended in a chance conversation. John solved the structure in collaboration with Jimin Wang, a postdoctoral fellow in Tom's group.

Shortly after this enterprise got under way, I learned that Anders Liljas and his colleagues at Lund University had been working on the same molecule for far longer than we had. Anders was characteristically generous in his

response to the news that we had entered the lists. The two groups solved the structure at about the same time, and we published back to back (25, 26).

A year or so later, I went on Scandinavian tour in which I visited both the Liljas laboratory at Lund University, where I served as the outside (Ph.D.) examiner for Artur Aevarsson, the first author on the Lund EF-G paper, and the group at Aarhus University led by Jens Nyborg that had just solved the structure of the EF-Tu-tRNA-GTP complex. The topic of my lecture at Aarhus was the structure of EF-G, and at the end of my lecture, a graduate student in the back row raised his hand and said (more or less), "EF-G looks just like the (EF-Tu) ternary complex." Immediately after the lecture, we went to a computer graphics terminal to see if he was right. I managed to download the Protein Data Bank (PDB) file for EF-G from home, which gave the people at Aarhus the illusion that I might be competent. The student in question, Poul Nissen (see below), then deftly did the superimposition required, which confirmed his assertion (27).

The similarity between the overall shapes of EF-G and the EF-Tu ternary complex is indeed striking, but I am still not sure I understand why it is that way. Long ago, Harry Noller and I had a conversation in which we agreed that the only thing we had really learned for sure from this discovery is that the Deity has a sense of humor. It seemed obvious that the Deity had allowed us to discover this similarity because he/she thought it would be amusing to watch us invent theories for elongation that would rationalize it. I hope he/she had a good laugh.

In the years that followed, members of my group solved several other crystal structures. Huijing Shi re-determined the crystal structure of yeast phenylalanine tRNA, which had provided the basis for all discussions of RNA conformation since it was first solved in the mid-1970s (28). Interestingly, at almost exactly the same time, Daniela Rhodes did the same thing at the MRC for the same reason (29). Advances in data collection and structure refinement technology guaranteed that a crystal structure obtained for Phe-tRNA in 2000 would be superior to its predecessors both in resolution and accuracy. Somewhat later, Szilvia Szep obtained a crystal structure for a small RNA motif she decided to call a "hook-turn" (30). Her structure was a fine example of something that has often happened to other RNA crystallographers. The RNA oligonucleotide she crystallized adopted a conformation *in crystallo* that was completely different from the one we had set out to investigate. The last such venture was carried out by Helen Merianos, who obtained a structure for ribosomal protein S8 bound to the sequence within its own mRNA with which it interacts to suppress its translation (31). None of these projects would have been possible had we not had access both to the facilities in the CSB and to its many experienced users, *e.g.* Jimin Wang, who generously advised us when problems arose, as they always do.

Ribosomes

From the mid-1980s on, ribosome crystallography was a recurrent topic for lunchtime conversations between Tom Steitz and me. Initially, I hesitated to get involved with ribosome crystallography both because I was so deeply enmeshed in other projects and because I was not comfortable competing with Ada Yonath. However, by the early 1990s, I began to feel that if something was not done by somebody somewhere, I might go to my grave ignorant of what the ribosome looks like. We agreed that if Tom were to find a postdoctoral fellow willing to work on the problem, my group would help with the biochemistry. By that time, Ada had produced ribosome crystals that diffract to atomic resolution, and thus, the issue was no longer crystallization, but rather phasing the diffraction patterns produced by ribosome crystals.

It took a while for Tom to find the requisite postdoctoral fellow because most people are unwilling to risk their careers on enterprises as dicey as the ribosome project then appeared to be. From the point of view of a postdoctoral fellow, there were (at least) two ways the enterprise could fail. On the one hand, it might turn out that the technology of the day was not powerful enough to allow anyone to phase ribosome diffraction patterns. On the other hand, if the problem could be solved, there was always the possibility that Ada or someone else would solve it first. The personnel problem was solved in the fall of 1995, when Nenad Ban joined Tom's group, eager to risk all on the ribosome. By the end of the year, we had crystals of the large ribosomal subunit from *Haloarcula marismortui* identical to those that Ada had shown can diffract to high resolution (32).

Two phasing strategies were pursued in parallel: molecular replacement and multiple isomorphous replacement (MIR). Earlier that year at a ribosome conference in Victoria, British Columbia, I had heard Joachim Frank describe the three-dimensional EM reconstructions of ribosomes embedded in vitreous ice that his group had just produced, which were leagues better than anything reported previously (33). It seemed likely that the low resolution reflections of the diffraction patterns produced by ribosome crystals could be phased by molecular replacement starting with models of that quality. If that could be done, it would be possible to locate the sites where heavy

atom compounds bind in ribosome crystals by difference Fourier methods, which work no matter how many sites there are, rather than by difference Patterson methods, which may fail if the number of sites is large, which it was likely to be for ribosome crystals in most cases. Our MIR strategy was also a low resolution strategy. It focused on heavy metal cluster compounds, which at low resolution have the same effect on diffraction patterns as "super atoms" of ultra high atomic number. The low resolution data sets needed could be collected using the protein crystallography stations installed on the bending magnet beamlines at the National Synchrotron Light Source at the BNL, which we could access whenever we wanted, more or less, because they were in low demand. By early 1996, Nenad had obtained his first heavy atom derivative. Because the particular heavy atom cluster compound he used to create that derivative happens to bind predominately at a single site per asymmetric unit, he had been able to determine the location of that site in the unit cell using conventional difference Patterson methods. It pays to be lucky!

Shortly thereafter, we encountered a problem that held us up for over a year. The crystals we were working with, which were grown in buffers that were nearly saturated solutions of monovalent salt, are very sensitive to ionic strength. In salt solutions just below saturation, the crystals that grow belong to the space group $C222_1$, but if the salt concentration is reduced by as little as 10%, a slightly different set of intersubunit contacts are favored, and pseudo-merohedrally twinned crystals are produced that belong to the space group $P2_1$. Preformed crystals can be made to switch back and forth between the two crystal forms by soaking them in salt solutions of the appropriate concentration. Not only are the accompanying changes in unit cell dimensions very small, but also the crystals show no signs of distress as they change space group. To make matters worse, the diffraction patterns produced by the two types of crystals have the same systematic absences, and because of the twinning, they also have the same rotational symmetry. The only things that are not the same in their diffraction patterns are the relative intensities of reflections. Until we understood what was going on, we were stymied by our inability to obtain reproducible data.

By the first of the year 1998, two new postdoctoral fellows in Tom's group, Poul Nissen and Jeffrey Hansen, had joined the fray, and by the middle of 1998, a 9 Å resolution electron density map had been produced (34). Crystallographic electron density maps having resolutions that low are seldom published, but this one really did deserve such notice. The electron density distribution displayed in that paper looked the way EM had taught us large ribosomal subunits should look, and it included many features that could be unambiguously identified as A-form double helix, all of which was very encouraging. More important, we could show that the heavy atom positions obtained for our derivatives by difference Patterson methods were identical to those revealed by difference Fourier methods using the set of low resolution phases that had been obtained by molecular replacement, starting with a cryo-EM model for the subunit produced by Joachim Frank and his colleagues. Thus, the ribosome phase problem had been solved at last. This paper earned Tom an all-expenses-paid trip to Stockholm in 2009, as it should have done. (Further details may be found in Tom's Nobel lecture.)

In the fall of 1999, the group obtained the first electron density maps with resolutions high enough so that sequence could be fit into them ($\sim$ 3 Å). I remember wondering when I first saw them how they would ever be interpreted, and indeed, it turned out to be a huge job. Map fitting was an all-hands-on-deck effort that went on for months, and the postdoctoral fellows were so desperate that they let me fit RNA sequence into the part of the map that turned out to represent domain I of 23 S rRNA. I had a great time doing it, and I am grateful to Nenad and Poul for their patience in instructing me about the mysteries of O.

An atomic resolution structure of the *H. marismortui* large ribosomal subunit was published in August 2000 (Fig. 4) (35, 36). A few weeks later, two structures appeared for the small ribosomal subunit from *T. thermophilus*, the second one being higher in resolution and much more accurately interpreted (37) than the first (38). The quest for the structure of the ribosome, which had motivated me for most of my career, had come to a successful conclusion, and I had been lucky enough to participate in its final denouement.

Much progress has been made since 2000 in obtaining the large number of atomic resolution structures that will be needed to fully elucidate the mechanism of protein synthesis. Structures are now available both for ribosomal subunits and for 70 S ribosomes from several prokaryotic species, as well as innumerable structures for these particles with substrates, substrate analogs, inhibitors, and protein factors bound (see Ref. 39), and the first eukaryotic ribosome structures have now made their way into the PDB (40, 41). In addition, many of the gaps that still exist in the set of crystal structures available have been filled by lower resolution EM reconstructions (see Ref. 42). Thus,

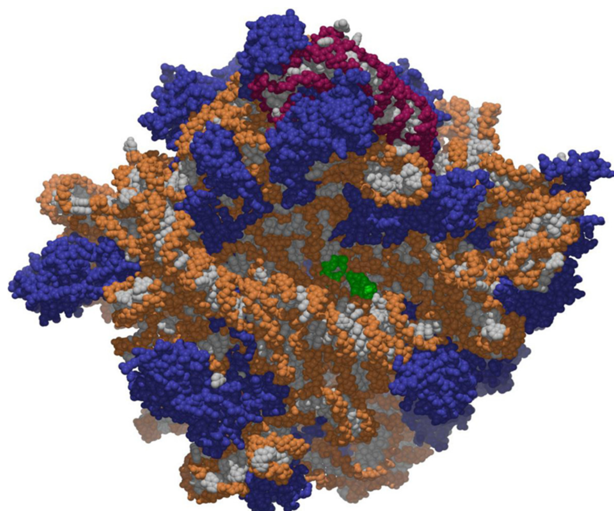

FIGURE 4. **Space-filling model of the large ribosomal subunit from *H. marismortui*.** The subunit interface side of the subunit is shown oriented so that the bottom of its active site cleft can be seen. A transition state analog (*green*) is shown bound in the peptidyl transferase center. Proteins are *dark blue*. RNA bases are *gray*. The backbone of 23 S rRNA is *brown*, and the backbone of 5 S rRNA is *purple*. This figure is derived from Ref. 36, but this version of it was produced specially for me by Professor Poul Nissen, whose assistance I gratefully acknowledge.

in little more than a decade, we have gone from abject poverty to riches beyond the dreams of avarice.

The Yale ribosome group has contributed its fair share to the avalanche of ribosome structures that have been deposited in the PDB since 2000. Members of my group, notably Neil Voss, contributed to the analyses that were done on our large subunit structure, which is still the highest resolution ribosome structure available (*e.g.* Ref. 43), and we helped with the elucidation of the mechanism of the peptidyl transferase reaction (*e.g.* Ref. 44). Members of my group (Susan Schroeder and Guliz Gurel) also collaborated with members of Tom's group, including Daqi Tu, whom we shared, in determining the structures of the complexes that anti-ribosomal antibiotics form with the large subunit.

The antibiotics that act on the large ribosomal subunit are all enzyme inhibitors, similar in character to the small organic molecules that inhibit more conventional enzymes. Almost all of them block protein synthesis by competing with ribosomal substrates, products, or protein factors for access to binding sites. The result, which is either a reversible cessation of bacterial growth or outright cell death, varies from one antibiotic to the next for reasons that are poorly understood in most cases. What makes the anti-ribosomal antibiotics intriguing biochemically is 1) that almost all of them bind to sites composed entirely of RNA and 2) that many of them are species-specific. Their specificity is remarkable because the ribosomes from all species are homologous, and their structures are nowhere more highly conserved than in their active sites, which is where antibiotics invariably bind. The structures and mutational studies we executed did cast some light on this paradox (45, 46).

As soon as we had a look at our first ribosome-antibiotic structures, we realized that the ribosome offers wonderful opportunities for structure-based drug design (47). It seemed highly likely that a drug discovery program based on our large subunit structures could result in the synthesis of novel antibiotics that are effective against drug-resistant pathogens. For that reason, a company called Rib-X Pharmaceuticals was founded in 2001 to exploit the Yale structures. Many friends and colleagues contributed to the founding of the company or got involved shortly thereafter, *e.g.* Tom (who was the prime mover), Susan Froshauer, Venki Ramakrishnan, Harry Noller, Bill Jorgensen, and John Abelson.

For-profit companies like Rib-X inhabit a universe quite different from the one familiar to academic scientists, but I find its challenges no less interesting. I am happy to report that the claim we made to the investors we courted in 2001–2002, namely that Rib-X would develop new antibiotics, has been fully vindicated, thanks both to the hard work of the many able scientists at Rib-X and to the good sense of their managers. It remains to be seen if the company succeeds commercially, but there are reasons for being optimistic on that front, too, despite the ugliness of the current economic environment.

The End Game

The older I got, the more irritating I found the NIH grant application circus. As luck would have it, both of the grants that supported my group were due for renewal in 2009, and as that deadline approached, I found the idea of letting them both terminate forever more and more attractive. In addition, some who read this article may be stunned to learn that their colleagues in chemistry departments actually have to teach for a living. The standard load is two term-long courses per year, one of which is usually a large enrollment course for undergraduates. As the years wore on, I was finding it increasingly difficult to develop the enthusiasm for freshman chemistry that I owed the students compelled to take it from me. Finally, I had been telling my colleagues for years that it is an abuse of the privilege of tenure for elderly faculty to hang on to the bitter end, not least because there are no 70-year-old scientists so wonderful that a 35-year-old scientist who is better cannot be found. So, in 2008, I sent my chairman a letter in which I promised to retire on July 1, 2010, which is

what I did. Others in similar circumstances should do the same.

Concluding Comments

Why did things work out for me as well as they did? Luck explains a lot of it. I have already alluded to the happy consequences that the accident of the date of my birth had on the rest of my life. Furthermore, most of the opportunities I took advantage of along the way came my way by chance, rather than as a result of any planning on my part. The fact of the matter is that rewards are distributed so nonlinearly in science that no one can hope to have a good career simply on the basis of talent alone; you have to be lucky at least once or twice.

There is only one thing you can do to load the dice in your favor, and that is to work at institutions where first-class science is being done. That is what I did. I spent my entire career in environments where I was surrounded by outstanding colleagues and mentored/guided by people like Jim Watson and later Fred Richards. In fact, we all owe more than we might like to admit to the institutions in which we work. For example, the reputation of a university for excellence is much more likely to attract graduate students than the prospect of working for a specific member of its faculty, and as everyone knows, it is almost impossible to have a career in academic science that amounts to anything without good students. So my complaints about computing at Yale not withstanding, I owe Yale a lot, and I happily acknowledge the debt I also owe to Harvard, the University of Geneva, and the MRC.

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