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Unraveling the Structure of the Ribosome (Nobel Lecture)**

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Autobiography. From Chidambaram to Cambridge: A Life in Science

Childhood

I was born in 1952 in Chidambaram, an ancient temple town in Tamil Nadu best known for its temple of Nataraja, the lord of dance. When I was born, my father, C. V. Ramakrishnan, was away on a postdoctoral fellowship in Madison, Wisconsin, with the famous enzymologist David Green. Because he came from a poor family, he did not think that he could support my mother and me on his postdoctoral income, so he went alone. I often joke that but for this, I would have been born in Madison and could have gone on to become President of the United States. In fact, I first saw him when I was about six months old. My mother, R. Rajalakshmi, taught at Annamalai University in Chidambaram, and during the day, I was well cared for by aunts and grandparents in the usual way of an extended Indian family. When I was about a year and a half, my father left again, this time with my mother, to go to Ottawa on a National Research Council fellowship. They returned a little over a year later, and during their absence I was brought up by my grandmother and my aunt Gomathi, to whom I remain close to this day.

When I was three, my parents moved to Baroda (now appropriately called by its Gujarati name of Vadodara, which refers to the abundance of banyan trees that the city used to have), where my father was appointed at an unusually young age to head a new department of biochemistry at the Maharaja Sayajirao (M.S.) University of Baroda. When he started the department, there was just some empty lab space with no equipment or people. He managed to acquire a low-speed table-top centrifuge, and would get blocks of ice from a nearby ice factory, crush them, and place them around the centrifuge so that his samples would remain cold during enzyme purification. With this setup he managed to publish two papers in *Nature* in quick succession. Within a few years, the department was well established in both teaching and research, and equipped with instruments, a cold room and an animal house.

Unusually for an Indian man of his generation, my father, being aware of my mother's intellectual abilities, encouraged her to go abroad by herself to obtain a Ph.D. She obtained a fellowship at McGill University to do a Ph.D. in psychology, where one of her mentors was the famous psychologist Donald Hebb, whose theories presaged modern ideas about synaptic plasticity as a basis for memory and behavior. Probably because she felt guilty about leaving my father and me behind, she finished her Ph.D. in under 18 months, which must be something of a record. When she returned, she could not find a suitable position in the Psychology Department in Baroda. Instead she used her analytical skills to help my father in his research, working initially as a CSIR pool officer, which was a temporary scheme by the Government of India to support scientists returning from abroad. This was the beginning of a lifelong collaboration in their work. My childhood and adolescence were filled with visiting scientists from both India and abroad, many of whom would stay with us. A life of science struck me as being both interesting and particularly international in its character.

My move to Baroda was something of a culture shock initially because until the age of three I only spoke Tamil, a language that I unfortunately no longer speak well. One of my earliest memories is of standing in a playground not being able to understand a word of the Gujarati the children were speaking. This feeling of being an outsider has remained with me for much of my life as my career has taken me to various countries. Because my parents did not speak Gujarati either, they enrolled me in what was then the only English language school in town, the Convent of Jesus and Mary School, which was located next to a large military base. After kindergarten, my mother persuaded the school to let me skip a grade. Shortly after my sister Lalita was born in 1959, my family went to Adelaide, Australia in 1960–61 where I studied in fourth and part of fifth grades. I remember the year in Adelaide as one of the most carefree years of my childhood, and returned with an Australian accent that my former schoolmates could hardly understand. Because of the six month difference between the Australian and Indian school years, I effectively again skipped a grade on my return. The rest of my schooling was at the Convent School. By that time, there were other English schools in Baroda, and the nuns who ran the school decided to convert it to a girls' school and no longer admit boys. They allowed those boys who had enrolled to stay on,

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but by attrition, our class kept losing boys, so there was a roughly four to one ratio of girls to boys when I graduated. Perhaps because of this and the fact that my mother and sister both went into science, I have felt perfectly comfortable among women even when I am the only male present, and there have been times when my lab has consisted almost entirely of women.

During the 7th to 9th grades, I dropped from being at or near the top of my class to being in the bottom third. Rather than studying, I spent my time playing and reading novels and other extracurricular books. Luckily, in my last two years I had a dedicated science and mathematics teacher, T.C. Patel (Figure 1), who made those subjects come alive. A strict

Figure 1. Early teachers. Top row: T. C. Patel, high school math and science teacher. S. D. Manerikar, mathematics professor in Baroda University. Bottom row: physics professors S. K. Shah and H. S. Desai, in Baroda University.

disciplinarian, he nevertheless had a twinkle in his eye as he would expose us to clever ideas and difficult problems. This sparked my interest in my studies again, and I graduated second in my class overall despite the fact that I did very poorly in Hindi, a language that I never managed to learn well.

Choosing Basic Science

By the time they finished high school, students in India were separated, as they are in England but not in America, into those who are going into science, medicine or engineering, and those who plan to study the arts or humanities. Although I liked literature and did well in my English class, studying English was never really an option I considered seriously, especially given the scientific environment in which I grew up. Additionally, the cultural climate in India makes it difficult for good students to choose to study the humanities unless they are particularly strong willed, because parents are too often opposed to what they see as a risky career choice. Accordingly, I enrolled in the pre-science course at my local university, the M.S. University of Baroda. This was a one-year preparatory course before students chose to go into medicine, engineering or basic science. The pre-science course had an excellent curriculum in both physics and mathematics, largely due to forward thinking faculty in those departments. However, the teaching of botany and zoology was very old-fashioned and involved memorization of lots of facts in a relatively unconnected and tedious way. As a result, I was not particularly interested in the life sciences at that stage.

A critical decision that students have to make after their pre-science year is whether to go into medicine or engineering. Generally, those students who did not get accepted into either of these went into basic sciences as a last resort. My mother, however, had just become aware of the National Science Talent Search Scholarship by the Government of India, which was modeled after the Westinghouse (now Intel) Science Talent scholarships in the USA. A condition was that the recipient had to major in a basic science. She encouraged me to take the scholarship exam, and arranged for me to do the required research project with a colleague of hers in the Biochemistry Department on quantifying nitrogen fixation by leguminous plants, which was somewhat ironic given my general apathy towards biology courses.

At the end of the year, I also took national entrance exams for the famous Indian Institutes of Technology (IITs) and for the Christian Medical College in Vellore, one of the finest medical schools in India, but which had a very small quota for males since it was founded to train female doctors. I did not do well enough to qualify for admission to either institution. However, as a result of doing well in my university exams, I was offered admission to study medicine in Baroda. In the meantime, however, I was offered the National Science Talent Scholarship. I had made an agreement with my father, who wanted me to study medicine, that if I was awarded this scholarship I could choose to study basic science. That decided, there was the further question of where to do my undergraduate studies. I briefly considered going to Madras, which would have reconnected me with my Tamil roots, but a faculty member in the physics department in Baroda, S. K. Shah (Figure 1), told me about a brand new curriculum they were introducing for their undergraduate course. It began with the Berkeley Physics Course, and was supplemented by the Feynman Lectures on Physics before moving on to more specialized areas. I therefore decided to enroll in the B.Sc. course in physics in Baroda, my hometown. Since I was only 16 when I began this course, I sensed that my parents, especially my father, were relieved that I was not leaving home at an age when they felt I was not sufficiently mature emotionally.

My teachers in physics, especially S. K. Shah and H. S. Desai, were very excited to be teaching the new curriculum for the first time, and their enthusiasm was infectious. I also had several excellent mathematics teachers, including the scholarly S.D. Manerikar, who discarded our exam-oriented

Indian textbooks and taught us from books like Hardy's *A Course of Pure Mathematics* and Courant's textbooks of calculus. It was during this time that I first met Sudhir Trivedi, who has gone on to a successful career in applied physics in the USA and become a lifelong family friend. When we had a boring class, we would often skip it. Once he and I sat near an open window and decided to jump out of it as soon as attendance was taken to go off and have tea and snacks at a nearby restaurant. His jump created a loud thud so I could not follow because the professor was staring directly at me.

Towards the end of my undergraduate studies I had to decide where to go to graduate school. The normal route for science students was to do a master's at some university in India before thinking of going abroad. As a Science Talent Scholar who was doing well, I would have been accepted almost anywhere. However, my parents were doing a short sabbatical at the University of Illinois in Urbana at this time, so it was tempting to spend the summer with them and go on to graduate school in the United States. By the time I applied, it was too late to take the GRE and without it almost no universities would consider my application. The University of Illinois accepted me into their graduate program in physics initially, but when they found out I was not yet 19 years old and had taken almost no non-science courses, they changed their minds and said I would have to enroll as an undergraduate! Needless to say, this was not an option I was prepared to consider, not least because it would have been impossible financially. At about this time, my chairman N. S. Pandya brought to my attention a letter from the physics department at Ohio University, which said they were looking for qualified students for their graduate program. I wrote them a letter of inquiry and soon afterwards was accepted with a fellowship. I was living alone when the acceptance arrived, and was absolutely thrilled to be going to graduate school in the USA, a land I associated with many of the great scientists whose textbooks I had studied, including Feynman, Purcell and others. I arrived in America a month after my 19th birthday, and spent the summer in Champaign-Urbana with my parents. I sat in on a number of mathematics and physics courses there, which helped ensure that I did not have serious deficiencies in my readiness for graduate school.

Graduate School in the USA

When I got to graduate school in Ohio, I was surprised to see that over half of our class consisted of foreigners, many of them from India. I passed the obligatory comprehensive exam after two years of coursework, and then chose to work in solid-state theory with Tomoyasu Tanaka. For my proposal, I had considered doing some theoretical work on biological systems, but since neither he nor I knew any biology, this did not go anywhere. The problem I took on was to look at ferroelectric phase transitions in potassium dihydrogen phosphate. This was a particularly difficult time for me, since I had no feel for the problem or even what the basic questions were. It was the first time in many years that I felt I had chosen the wrong field. At the same time, I found myself tremendously interested by the articles in biology in *Scientific American*, to which I have subscribed off and on through the years. It appeared that hardly a month went by without a major breakthrough in the life sciences, whereas physics was having a hard time making any fundamental progress. Certainly I felt that if I continued in physics, I would be doing boring and tedious calculations rather than making really interesting advances. The result was that I felt so frustrated that I withdrew from my thesis work and spent an inordinate amount of time on extracurricular activities. I went hiking and hopped on freight trains with my good friend and class mate Sudhir Kaicker, learned about western classical music from another friend, Anthony Grimaldi, played on the chess team, read literature, and went to concerts. In short, I did everything except make progress on my work. Tomoyasu was far too kind and patient, but even he would get worried every few months and ask how I was getting on. I was too embarrassed to tell him that I wasn't getting on at all! I often joke that if I had graduate students like me, I'd fire them! In hindsight, I feel that given my aptitude and tendencies, I made a mistake in choosing theory over experiment for my thesis work, and I might have done better under someone like Ron Cappelletti (now at NIST), who was not only a fine experimentalist but also tougher and less likely to tolerate an irresponsible student. Interestingly, I later became good friends with Ron.

It was during this time that I met Vera Rosenberry, who was majoring in painting and was introduced to me by mutual friends because, unusually for the early 1970s in Ohio, we were both vegetarian. After an intermittent courtship that lasted only 11 months in total, we were married in 1975. She has been my companion and friend ever since, and has not only done most of the work of raising our children but uprooted herself many times to move with me all over the USA and to England. The emotional support and stable home environment she provided has been invaluable to me and my work. During that time, in addition to painting, she also became a children's book writer and illustrator, and has published over 30 books.

After my marriage at the age of 23, I was suddenly no longer alone but had a wife and a five-year-old stepdaughter, Tanya Kapka. This sudden change in my responsibilities made me realize that I had to get on with my career. I produced a passable thesis in the next year and obtained a Ph.D. in physics in 1976 just a month before our son Raman was born. But by that time I had already decided I was going to switch to biology.

Transition to Biology

Since I hardly knew any biology, I felt I needed formal training of some sort. I could go to graduate school again, with the option of getting a second Ph.D. or go to medical school, which was ironic since I had turned down the opportunity to do precisely that when I was younger. I took the MCAT (a nationwide medical college entrance exam) but despite scoring in the 99th percentile in all the subjects, I only got one interview (at Yale) because I was not a U.S. citizen or even a permanent resident at that point. During that interview, I said I was mainly interested in research and not

interested in practicing medicine since I felt the US medical system involved a fundamental conflict of interest between doctor and patient. Needless to say, I was not selected. However, I had also written to a number of graduate programs. Many of them said that they would not accept someone who already had a Ph.D. The chairman of the Molecular Biophysics and Biochemistry (MB&B) department at Yale, Franklin Hutchinson, wrote to me saying that while they could not take me as a graduate student, he would circulate my CV to faculty members for a potential postdoctoral position. Two of them responded: One was Don Engelman, and the other, ironically, was Tom Steitz, with whom I shared the Nobel prize. Although I found their work very interesting, I thought doing a postdoc directly from a degree in physics would leave me with too narrow a background in biology to be an effective scientist. So when three schools accepted me into their graduate program, I chose to go to the University of California, San Diego (UCSD), partly because of its large and diverse faculty and partly because La Jolla seemed an easy place in which to bring up young children.

During the first year, I did several lab rotations in biology and took as many undergraduate courses as I could possibly manage, including genetics, taught by Dan Lindsley, a well-known Drosophila geneticist, and biochemistry, where I was inspired by the brilliant and enthusiastic lectures of Paul Price. During a rotation project in Milton Saier's lab, which was studying sugar transporters in bacteria, I was asked to measure the rate of sugar uptake in various mutants. The idea was to pipette 20 μL of a stock of ^{14}C glucose into the tubes containing cultures at time zero, and then withdraw aliquots at regular intervals and measure the amount of glucose uptake by filter binding. I asked how they measured such a small volume as 20 μL. The technician showed me a micropipettor and how to set the volume and use it. We started, but as soon as I had plunged the micropipettor into the radioactive glucose solution, she screamed at me, "What do you think you're doing? You have to use TIPS!" This episode has always made me more tolerant when my students do something out of ignorance resulting from lack of complete communication.

My first year in UCSD was tremendously exciting. For the first time in my life, I was at a university that was at the forefront of international research. I also made some wonderful friends, including Robert Anholt, now a professor in North Carolina who works on olfaction, and Mark Troll, a brilliant physical chemist who many years later married my sister.

In my second year, I settled down to do research in Mauricio Montal's lab. Mauricio had developed an ingenious method of incorporating conducting channels into lipid bilayers formed by bringing together two defined monolayers, and was thus doing single molecule biophysics at a time when nobody called it that. Around this time, however, I read an article in Scientific American by Don Engelman and Peter Moore (Figure 2) about their ribosome work, and became interested in it. It also struck me that there was no longer any reason to continue on to obtain a second Ph.D. because I felt I had acquired the background I needed. I therefore wrote to Don Engelman, one of the two people from Yale who had responded to me earlier. Don was interested in membrane proteins, a subject I was already working on in Mauricio's lab. Don wrote back and said that he and Peter had a position open on their ribosome project, and I could always work on membrane-related projects once I got there. Peter arranged to meet me in San Diego in early 1978 and offered me a postdoctoral position soon afterwards. Thus began my lifelong interest in ribosomes.

Figure 2. Peter Moore (left), postdoctoral and lifelong mentor who introduced me to ribosomes. Stephen White (right) who encouraged me to get into crystallography of ribosomal proteins.

Postdoctoral Work in Peter Moore's Lab at Yale

Peter Moore ran a very small lab with generally no more than four or five people. Among them was Betty Rennie (now Freeborn), who taught all of the lab members how to purify ribosomes, reconstitute them and assay them. The specialized methods I learned in Peter's lab were invaluable to me twenty years later when I started tackling the structure of the 30S subunit that led to the Nobel Prize. Peter has been a lifelong role model. He is brilliant, rigorous, fair, straightforward and honest with his opinions, and has been a supportive mentor all my life. In his lab I participated in the long term project of mapping the spatial location of the proteins in the 30S subunit, which involved reconstituting ribosomes in which a specific pair of proteins were replaced by their deuterated counterparts. This was followed by small-angle neutron scattering experiments at Brookhaven National Laboratory, which would yield the distance between the centers of mass of the two deuterated proteins. I managed to map about a third of the proteins during my stay there, as well as contribute to the use of Bayesian methods to obtain the best possible estimates from the data.

Postdoctoral work combined with a family with two young children did not leave me much time to socialize with fellow students or postdocs. But we did have a number of good friends because we lived in Yale housing. I also remember these years as being particularly happy, since I was finally making progress on a real research project and felt that my career was finally beginning to take shape. This feeling

quickly ran into reality when I applied for a large number of faculty positions during my final year at Yale, and did not get a single interview. This was partly because of my rather unusual career path, the fact that my degrees were from less than first-rate institutions, and perhaps more importantly, universities did not know what to think of a physicist turned biologist using an esoteric technique like neutron scattering. I was beginning to get very discouraged when two openings finally materialized. One of these was a limited-term position at the National Bureau of Standards with Alex Wlodawer. The other was the result of a phone call by Don Engelman to Wally Koehler at Oak Ridge National Laboratory, who was looking for a biologist to hire for their new small-angle neutron facility. I chose the Oak Ridge position because it seemed more stable and permanent, and I was told that I could have a joint appointment in the Biology Division and be able to carry out my own research in addition to collaborative experiments using neutron scattering. I should have waited until the end of the school year to move, but instead chose to do so in February during an ice storm.

An Interlude in Oak Ridge

Soon after I arrived in Oak Ridge, I realized that I had completely misunderstood my position. The Biology Division saw me entirely as an employee of the neutron scattering facility, and would not commit any resources to support me, although they gave me some space in an empty lab. I tried to remain positive and told my boss Wally Koehler that I could apply for NIH grants to fund my own research. He replied that it would look bad if I got external funding while senior scientists in the Biology Division were struggling, and moreover he had not hired me to be a biologist but to attract biologists to the use of neutrons. I found this attitude insulting and ignorant of both the way biology is done and the rather peripheral role that neutrons play in tackling important biological problems. As a result, I began looking for alternatives within a month after arriving in Oak Ridge, despite the fact that we had foolishly just bought a house.

While I was looking for a job, I talked to various people in the Biology Division and identified several projects where neutron scattering could provide some useful information. In the Division, I shared an office with Rose Feldman, a longstanding technician who worked for Salil Niyogi. Rose was my psychological support in what would otherwise have been a very difficult year. Moreover, I began collaborations with Mark Donnelly and Rick Wobbe, who both became lifelong family friends along with Mark's wife Veta Bonnewell. I have never made so many longstanding friends in such a short time. One of the most useful collaborations was work on the nucleosome with Gerry Bunick, Ed Uberbacher and Don and Ada Olins. This sparked an interest in chromatin that continued until 1998, when I gave it up to concentrate entirely on the ribosome.

Starting an Independent Career at Brookhaven

The Yale neutron effort on ribosomes was the direct result of instrumentation developed by Benno Schoenborn for the biological use of neutron crystallography and scattering at Brookhaven National Laboratory. When Benno learned of my situation in Oak Ridge, he told me to be patient and that a suitable staff scientist position would soon be available at Brookhaven that came with appropriate support and the freedom for independent research. I will always remain grateful to Benno for hiring me at Brookhaven and thereby rescuing me from scientific oblivion.

Personally, the move to Brookhaven was not easy. Vera and I liked Oak Ridge and the beautiful countryside around it. We were not enamored of the car-oriented commuting lifestyle of Long Island. But the job at Brookhaven was an excellent one in a department with fine colleagues, so we moved there in 1983. We sold our house in Oak Ridge for about 25% less than we had paid the year before and bought a house in East Patchogue, just outside the village of Bellport on the south shore of Long Island, about a 12-mile commute each way from the lab. There we became lifelong friends with Karen and Bruce Brunschwig. Bruce later played a crucial role in our ribosome effort by synthesizing a key derivative for us, osmium hexamine.

Unlike my experience in Oak Ridge, Brookhaven gave me exactly what they had promised. Benno said it would be nice if I did some experiments that used neutron scattering, but that they were broadminded and I could generally do what I wanted. They did advise me that if I wanted to get tenure, I could not simply continue what I had been doing in Peter's lab as a postdoc, but had to show independence. Nevertheless, my first experiment was on ribosomes, in which I tried to settle an emerging controversy about whether the proteins and RNA in the 30S subunit were asymmetrically distributed. This resulted in my first independent paper being a single author paper in *Science*. Since this was a decade before the internet, I wrote a letter to my father in India when it was accepted, and about a month later received his reply saying that he was glad I had made a good start, and that if I continued to work hard, I might some day even have a paper in *Nature*!

I also continued working on chromatin. The linker histone H1 binds to nucleosomes and helps to organize nucleosomes into a higher order structure, commonly known as the 30 nm filament. I wanted to try to use neutron scattering with deuterated histone H1 to determine its location in the intact filament. But it was not clear how one could go about getting deuterated histones, and I spent at least a year in a futile effort to grow Euglena gracilis in deuterated media to try to isolate histones from it.

At about this time, two crucial developments occurred. The first was that Steve White (Figure 2) joined me on the staff at Brookhaven. He had moved from the Max Planck Institute for Molecular Genetics in Berlin, where in Wittmann's department, he had been working on the crystal structures of several ribosomal proteins. Because of our shared interest in ribosomes, he very kindly offered to collaborate with me. Initially, I did not contribute much to the project because I was focused on getting my own projects

under way and collaborating with Wally Mangel on the conformation of plasminogen in solution. I began working with Steve by helping to grow large amounts of bacteria in a Yale fermentation facility, but when I saw the low yields that were obtained by purifying proteins from native ribosomes from Bacillus stearothermophilus, I felt that there had to be a better way. Indeed there was, and I could not have been in a better place to find it.

The second development was that at about this time, my colleagues Bill Studier and John Dunn (Figure 3) had sequenced the genome of the bacteriophage T7, and had

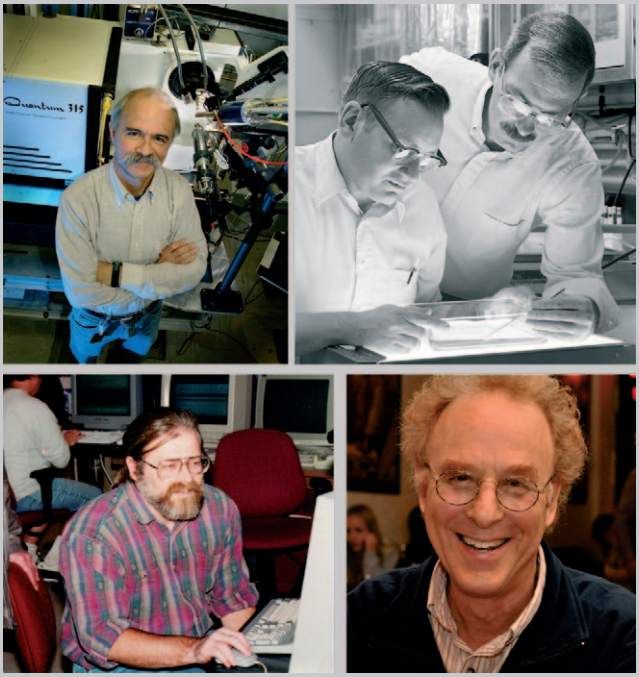

Figure 3. Brookhaven colleagues. Top: Bob Sweet, Bill Studier, and John Dunn. Bottom: Malcolm Capel and Bruce Brunschwig.

begun cloning a number of its genes. Importantly, because T7 RNA polymerase had its own promoter, they and their co-workers realized that they could overexpress target genes in E. coli using the T7 promoter and a cloned copy of T7 RNA polymerase under inducible control. They could also use T7 RNA polymerase to transcribe target RNAs. Their work has revolutionized biochemistry, and today there is scarcely a lab in the world that has not at some point used their system to make large quantities of protein in E. coli. John and Bill, as well as their associate Alan Rosenberg, and two plant geneticists, Ben and Frances Burr, took me under their wing and taught me all about cloning, making libraries, doing Southern blots, and a then new technique called PCR. The result was that I was one of the few structural biologists who was familiar with these tools at the time. Luckily, I had two wonderful technicians, Sue Ellen Gerchman and Vito Graziano, who were quick to learn these techniques as well, and apply them to our work. They were later joined by the energetic Helen Kycia. These tools allowed me to think about cloning the genes ab initio for the various ribosomal proteins that had been crystallized from native sources. This idea worked very well and the result was that material was no longer limiting. Moreover, in our chromatin work, we expressed histone H1 in *E. coli* and used that as a route for deuteration.

When I had been at Brookhaven for a few years, I was asked by the departmental tenure committee what I would do if they gave me tenure. I said I would probably stop doing neutron scattering and go away on sabbatical to learn crystallography because that was how I could answer the really important questions in my field. To my relief, they were quite enthusiastic about the idea and awarded me tenure. My colleague and friend John Dunn brought a large stick covered in aluminum foil to my home and presented the symbolic staff to me, saying, "Welcome to the tenured staff."

Steve had constantly been encouraging me to get interested in crystallography, saying that someone with my physics and small-angle scattering background would have no trouble learning it. He finally kick-started me into it by setting up, on my behalf, the first crystallization trials of the globular domain of a linker histone, GH5. Shortly afterwards, in the fall of 1988, I took the first Cold Spring Harbor Course in crystallography. In subsequent years, this course was attended by several well-known scientists including Rod Mackinnon and Art Horwich. During the following year, we obtained crystals of GH5 that diffracted well. By then, several of the ribosomal proteins that we had over-expressed had crystallized in previously identified conditions. So there were several crystallographic projects that needed to be carried out. I felt the best way to learn how to solve these structures was to go away on sabbatical and do nothing else but work on them. There was only one place I wanted to go for a sabbatical, the MRC Laboratory of Molecular Biology in Cambridge, England. There were several reasons for this: it was the birthplace of crystallography and many technical developments had occurred there; it had a huge reputation in molecular biology; the director at the time, Aaron Klug, was considered a giant in the field of chromatin structure; finally, Vera and I were both anglophiles and we liked the idea of spending a year in England. I therefore wrote a letter to Aaron Klug, pointing out that I had crystallized GH5 and would like to come for a year to learn enough crystallography to solve its structure. He wrote back an encouraging letter, and supported my application for a Guggenheim Fellowship, which covered half my salary while Brookhaven paid the other half.

At Brookhaven, Bob Sweet (Figure 3) taught me a great deal about data collection and crystallography in general. As a result, shortly before I went to Cambridge, Steve White and I collected data on ribosomal protein S5 using a previously identified gold derivative, and with Bob's encouragement and advice, Vito Graziano and I collected multiwavelength anomalous diffraction (MAD) data on crystals of selenomethionyl GH5. With the data in hand, I set off for Cambridge in late August, 1991, with Vera and our son Raman, who was then 15 years old, and our daughter Tanya, who had just graduated from Oberlin College and wanted to spend a couple of weeks touring England.

Sabbatical in Cambridge

Arrival at the MRC Laboratory of Molecular Biology (LMB) was a surprise in many ways. We had rented a large car at the airport in which to carry all our luggage and our bicycles. Driving on the left side of the road after an overnight flight from New York was challenging. When we got to the Addenbrookes Hospital site, I was lost and asked someone where the famous MRC Laboratory of Molecular Biology was, and to my great surprise, the first few people I asked had no idea! This reminded me of the story in Crick's autobiography *What Mad Pursuit* in which his taxi driver had no idea where the Cavendish lab was.

I already knew that the LMB was a crowded laboratory with shakers and refrigerators in the hallways, but coming from Brookhaven, where my department building had originally been a bowling alley in an army base, this lack of poshness did not bother me. When I arrived, my host John Finch, a Fellow of the Royal Society and longstanding colleague of Aaron's, told me that unfortunately they did not have a bench or desk for me to work at just yet. I rather naively told him that all I needed was a small corner in his lab. John politely smiled at this, and I found out a day later that he, a world-famous scientist, only had a desk and a small part of a lab bench! This was still a place where senior scientists did lab work and shared offices if they had one at all.

On my first full day there, I arrived around 9 am, and about an hour and a half later, John came by and asked me if I would like to go up to the canteen for coffee. I thought I had hardly done anything yet and declined, saying I didn't drink coffee. Again, John gave me one of his enigmatic smiles and a colleague who watched this exchange, said, "He hasn't learned our ways yet." As the days passed, I realized that these regular breaks from work to have meals or coffee or tea allowed scientists to talk informally together in the famous canteen on the top floor. It is also true that the human mind can only really concentrate for a couple of hours at a time, and these breaks re-energized people. The canteen was especially wonderful for a sabbatical visitor because I quickly got to know lots of scientists. Two of them, Daniela Rhodes and Kiyoshi Nagai, had labs adjacent to where I worked and were very welcoming and friendly. They have gone on to become lifelong friends and colleagues.

There were two other important lessons I learned at the LMB. I found that almost nobody there was working on routine problems just because they would lead to publishable results. Rather, they were trying to ask the most interesting questions in their field and then developing ways to address them. The other lesson was that even very famous scientists would ask questions at seminars that were often trivial to people in the field. It reinforced in me the feeling that ignorance is not something to be ashamed of, and that no question is too stupid to ask if you want to know the answer.

Although I had come with MAD data on GH5, Aaron thought that the structure of the protein by itself might not be interesting enough and wanted me to try to form and crystallize a complex of GH5 with DNA. The problem was that it was not a sequence-specific protein, and it wasn't even clear whether a duplex of DNA was an appropriate target. But being in awe of his reputation, I started working on it, sharing a bench with his brilliant postdoc, Wes Sundquist (Figure 4). After about a month, I realized that this was not

Figure 4. Utah colleagues. Dana Carroll (left), my chairman, Wes Sundquist (middle), and Chris Hill (right).

something that could be done on the time scale of my sabbatical, if ever. With some trepidation, I told Aaron why I thought this was not a useful approach and that I wanted to focus on solving the two structures using the data I had brought with me. Somewhat to my surprise, he readily agreed, and I think it increased his respect for me. When I stopped working on forming GH5–DNA complexes, I stopped doing any bench work for the rest of my sabbatical. My last gel was left unused on my bench, and it was with great amusement that Wes watched it slowly dry out over the next few months.

With the help of many people, including Phil Evans, Andrew Leslie and especially Paul McLaughlin, I learned the basic nuts and bolts of how to solve a structure. At a crucial point early in my stay, Alwyn Jones visited from Uppsala, and showed me how to start building a structure into maps using his program O. The result was that by the end of my sabbatical year, both structures were solved and eventually published in *Nature*. In the second of these, GH5, I had shown that one could treat MAD data as a special case of isomorphous replacement with anomalous scattering. This was the direct result of a suggestion of Eleanor Dodson's, who conveyed it to me through Phil Evans. This worked extremely well, and helped to change the way that MAD structures are solved in practice. I was particularly proud when only a few years after having taken the first Cold Spring Harbor crystallography course, I was invited to give a lecture in it because of my contributions to methodology.

Move to Utah

Upon my return to Brookhaven, apart from finishing our last neutron scattering experiment to locate H1 in the 30 nm chromatin filament, my lab focused almost entirely on the crystallography of ribosomal proteins and factors. However, my experience at the LMB made me unsatisfied with the environment at Brookhaven. Although I had excellent colleagues, the Department of Energy that ran the laboratory increasingly emphasized large projects over individual investigator-initiated research. It became very hard to recruit new staff scientists to the lab particularly in new areas of biology. The support offered to principal investigators was not sufficient to run even a modest sized group of a few people,

and grants, e.g. from the NIH, were not easy to get, partly because of the overhead costs charged by the lab, which were much higher than charged by many universities.

I wrote to Richard Henderson, the head of the Structural Studies Division at the LMB to ask if they had any openings for me. He wrote back a friendly letter saying they all liked me during my sabbatical but had no openings, so I should just stay in touch. Not knowing Richard, I simply took this to be a polite no. Meanwhile, my sabbatical bench mate, Wes Sundquist, was now an assistant professor in Utah and invited me to give a seminar. A few months later, his department asked if I would be interested in applying for a job there. I very much liked both the faculty at Utah and the spectacular location of Salt Lake City, nestled in a valley surrounded by beautiful mountains. There were a lot of people interested in ribosomes and RNA there, including Ray Gesteland, John Atkins, Brenda Bass, and Jim McCloskey. I also particularly liked Wes and the charismatic and ambitious crystallographer Chris Hill, who would be my immediate colleagues. When I was offered the job, I was staggered because they offered me a salary that was $20,000 (or 30%) more than I was making at the time, and felt they must be expecting something of me that I couldn't possibly deliver. I accepted the job, but a week later, I panicked at the thought of having to be totally reliant on external funding once my start-up had run out, so I actually called up Dana Carroll (Figure 4), the chairman, and said I was sorry but I couldn't come after all. They were understandably unhappy about this sudden waffling on my part, but fortunately kept the position open and allowed me some time to think about it. So after some agonizing, I decided to put aside my fears about funding and move to Utah.

The Biochemistry Department at the University of Utah was a small but dynamic department with a relatively young and ambitious faculty working on exciting problems. The department lived up to every promise it had made, and within a few months, I had settled in and got my lab running. Wes and Chris were wonderful and supportive colleagues, who occasionally acted as my psychiatrists whenever I would panic about failing. My chairman, Dana, became a close personal friend because we were similar in age and had common interests such as chamber music and hiking.

Bob Dutnall, who had done his Ph.D. at the LMB under Daniela Rhodes, joined me as a postdoc to work on chromatin modifying enzymes. We went on to solve the first structure of a histone acetyltransferase, Hat1, in collaboration with Rolf Sternglanz from Stony Brook. We were helped by a technician, Adrian Hahn. Except for Bob, and another postdoc, Mabel Ng, the lab was now focused on solving ribosomal protein structures. Bil Clemons joined the lab initially to work on ribosomal protein S15. Brian Wimberly had turned down a faculty position to do a second postdoc with me. He first solved a ribosomal protein structure, and then solved the structure of the first protein–RNA complex in the ribosome, that of L11 with a piece of RNA that binds to it. This part of the ribosome is also the target of antibiotics such as thiostrepton. The effort involved in this made me realize how much work it would take to solve all of the binary protein-RNA complexes in the ribosome, and how little we would learn about how the ribosome actually works from that effort. Even before coming to Utah, I had ideas of solving the structure of the ribosome, beginning with its small or 30S subunit. These ideas and their scientific consequences are outlined in my Nobel lecture and will not be discussed here. But how it happened from a human point of view is perhaps interesting.

My first task was to convince someone in the lab that this was a worthwhile project. Brian was cautious. Perhaps he knew too much about the difficulties, and also because it was his second postdoc, felt he couldn't take such a risk. So he decided to focus on solving the complex of protein S7 with its binding site on RNA. I made a friendly but wildly optimistic bet with him that we'd solve the entire 30S (including S7 of course) before he solved his complex. Another postdoc, Matt Firpo, worked on the problem for about a year, but had to leave when his funding ran out.

The project really took off when the two graduate students came on board. Bil Clemons was a smart and very ambitious student who fortunately was at a stage where he was willing to gamble on something potentially big. His eyes lit up at my suggestion and he immediately said he would take on the project. He worked on two small proteins to learn crystallography first before devoting his full attention to the 30S subunit. John McCutcheon also enthusiastically decided to devote full time to it. I knew I needed Brian Wimberly on board because of his encyclopedic knowledge of RNA structure and the literature on ribosomes, and as soon as we had made tangible progress, he too joined the team. With a capable technician to assist us, Joanna May, we were off and running.

As soon as we started, my insecurities about funding again set in. I could just imagine writing a grant application to NIH saying that we had no good crystals of the 30S subunit but had some ideas about how to get them, and that although a group had been working on good crystals of the 50S subunit for almost a decade, we had some ideas for how to solve our structure if we got good crystals. Having served on study sections myself, I could just imagine the peals of laughter that would go around the table as my application was considered. On the other hand, I knew that the LMB, where I had done my sabbatical, had a longstanding tradition of supporting exactly this kind of difficult but fundamentally important project. Apartment from funding issues, I felt I would have access to world leaders in crystallographic methodology who could help me if I ran into technical problems.

So I wrote again to Richard Henderson, who by that time had also become the director of the LMB, and we agreed that I would visit on my way to a ribosome meeting in Sweden. After my talk on ribosomal proteins, Richard and Tony Crowther (who was joint head of the division with Richard) chatted with me for a couple of hours on the "ribosome problem." They were interested in my ideas, what the competition was likely to be, what approaches had failed, what resolution one would have to reach to achieve a significant breakthrough in understanding and how long that was likely to take. The conversation was unlike any other job interview. There was no discussion of space, salary or equipment, just about science and ideas. At the time, I had no crystals; nevertheless Richard wrote shortly after my visit

saying they were interested in supporting me, and would let me know when they would have the additional space to accommodate me. Aaron, Daniela and Kiyoshi also expressed support for the idea of my move there. A few months later, Richard wrote again to say that indeed the space had materialized. I suddenly had to make what was one of the hardest decisions of my life: whether to gamble everything on going to the LMB and work exclusively on this project, which would involve taking a large salary cut and leaving our families (including our grown children) in the USA, or to continue working in Utah, where I would probably have to hedge my bets by working on safer projects simultaneously. In the end, I decided that the structure of the ribosome was the most important goal in my field, the time was ripe for an attack on it, and it would be a mistake to be distracted from it by other projects because there was only a narrow window of opportunity before other groups entered the field that had so long been dominated by just one person, Ada Yonath.

Most people thought that it would be insane to move to England staking all on this one risky project. Two people who encouraged me to go were Peter Moore and Steve Harrison. Both recognized from their own careers the ambition to solve a fundamental problem regardless of the challenges. While many in my family were ambivalent, my mother encouraged me to put aside my fears and go to Cambridge and give it a shot. Vera and I finally decided to leave Utah where we were very happy, take a 40% salary cut and move to the LMB. It was a very difficult moment for me when I had to tell Dana, Wes and Chris of my decision. I had to stay there for another year to work out the logistics of moving. During this time, I came to realize how truly wonderful my Utah colleagues were. They put aside any disappointment at my leaving and were both understanding of my decision and supportive of my efforts during my last year, helping in every possible way in our work. In one last hiccup, I almost didn't move to the LMB when I found I was going to lose John McCutcheon because he had personal reasons not to move, while during the student recruitment day in Cambridge I found myself unable to attract anyone to this effort. Fortunately, two people courageously agreed to join my lab at the LMB without ever having met me: Andrew Carter, who joined me from Oxford as a Ph.D. student, and Ditlev Brodersen, a postdoc who came highly recommended to me from Århus in Denmark, where I knew his supervisor Morten Kjeldgaard. Their joining me was a stroke of luck and meant we would have a viable team at the LMB.

With the decision to move to the LMB made, I decided to focus entirely on the 30S subunit. Within a few months we had crystals, and a few months later, we had cracked the problem of getting them to diffract well. This was largely due to John and Bil's willingness to try completely new approaches to purifying the 30S subunit and to their sheer dedication and hard work. We were also helped by Malcolm Capel's willingness to let us screen lots of our initial crystals at the NSLS at Brookhaven. Shortly before our move to the LMB, we collected several derivatives to 5–6 Å resolution.

Work at the MRC Laboratory of Molecular Biology

I moved to Cambridge in April of 1999, while Brian, Bil and Joanna stayed behind. The result was that I was able to make use of the LMB's computing resources to try several phasing runs in parallel, and send the maps to Utah where Brian and Bil would look at them. Because of the 7 hour time difference, we may have been the only group that actually speeded up to some extent as a result of a move. I suggested to Brian, who up to that point was focusing entirely on the RNA in the maps, that some of the tubes we were seeing were probably alpha helices of proteins. By the time I left that day, he told me he had identified a protein, S6, in the maps. The next morning, I was amazed when I came to the LMB to find out that he had identified all of the proteins of known structure in the maps! He said it was like eating potato chips: once he had identified one, he couldn't stop. With his knowledge of the protein-RNA interaction data, and his feeling for RNA folds, he went on to trace the entire central domain of the 30S subunit, and also identify a protein of previously unknown structure, S20. So only a few months after my move to Cambridge, with the rest of my lab still in Utah, we had made a major breakthrough. When I revealed our findings at the triennial ribosome meeting in Denmark in June, I could sense the shock in the audience, especially since virtually none of them knew we were working on the problem. Soon afterwards, our work was published in Nature in August, 1999 with much fanfare.

Although Tony, Kiyoshi, and Daniela were very welcoming to us, we had trouble settling into Cambridge. Vera and I were shocked by the rapidly increasing housing prices, and for a while despaired of ever finding a house we could actually buy since we kept being outbid on the many offers we made. When I was away on a synchrotron trip at Brookhaven, Vera found a house in Grantchester, a historic village just outside Cambridge that was within bicycling distance of both the city center and the LMB, and made a bid that was actually accepted. So we finally had a permanent home in Cambridge. By this time, Brian and Bil had moved from Utah, and we needed to focus on getting to high resolution.

Solving the 30S Subunit Structure

Getting to the high-resolution structure of the 30S subunit was beset with problems, which are described in the Nobel lecture. This was a particularly stressful time for me and my lab members (Figure 5). The Yale group of Tom Steitz, Peter Moore and their colleagues was making steady progress with their structure of the 50S subunit. More significantly for us, soon after we had decided to focus on the 30S subunit in Utah, I had found out that Ada Yonath, who had first crystallized the 50S subunit and had been working on determining its structure for over a decade, had now essentially switched to determining the 30S subunit structure using crystals obtained by a slightly different route. So instead of having a quiet niche to myself, we were in a flat-out race. Our progress slowed down because the limitations of the crystals meant that we could not collect data to high resolution at Brookhaven. The

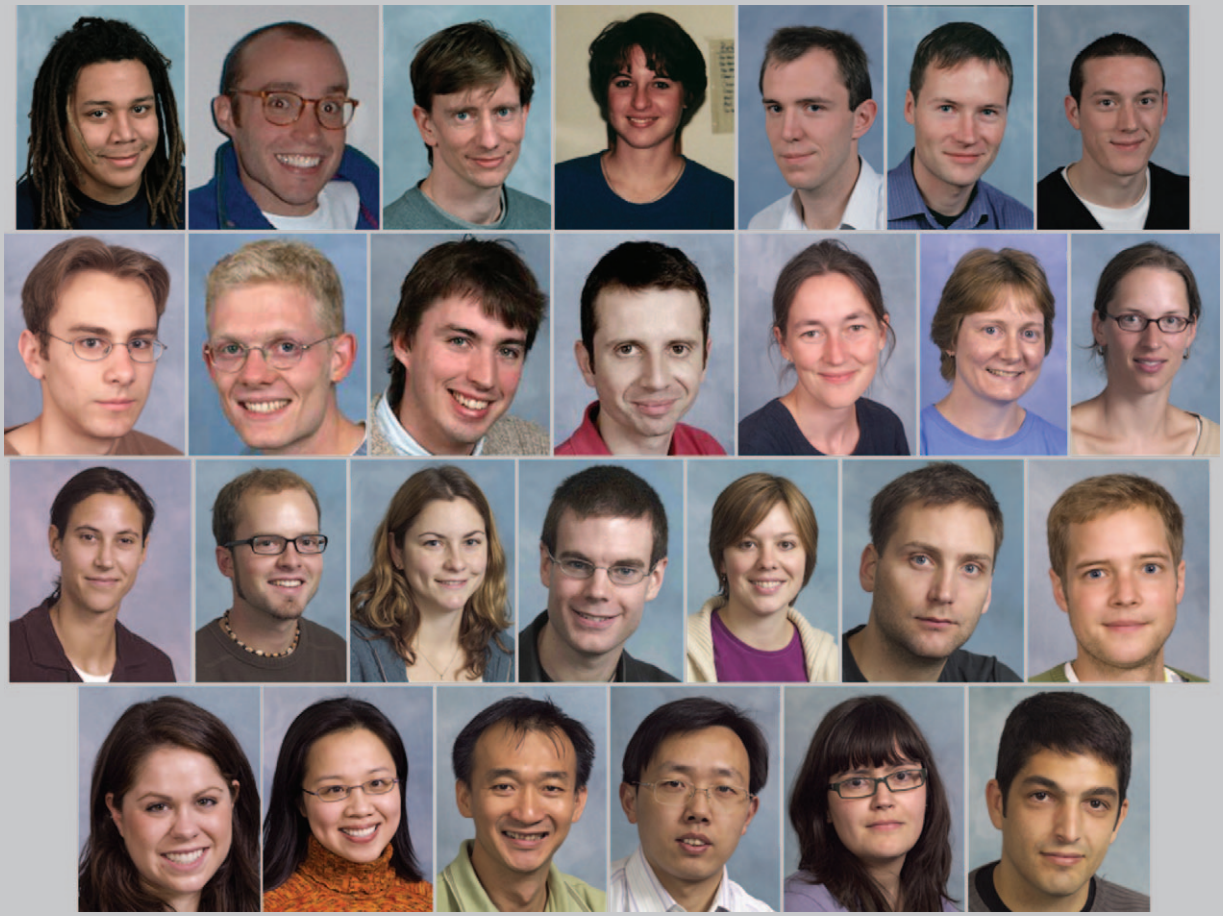

Figure 5. Members of lab (1996–2009) who contributed to the ribosome structure work in the order in which they joined. Row 1: Bil Clemons, John McCutcheon, Brian Wimberly, Joanna May, Andrew Carter, Ditlev Brodersen, Robert Morgan-Warren. Row 2: Mike Tarry, James Ogle, Frank Murphy, Reynald Gillet, Maria Selmer, Ann Kelley, Tina Daviter. Row 3: Sabine Petry, Albert Weixlbaumer, Christine Dunham, John Weir, Lori Passmore, Martin Schmeing, Caj Neubauer. Row 4: Rebecca Voorhees, Hong Jin, Leong Ng, Yong-Gui Gao, Kathrin Lang, Israel Sanchez.

one high intensity beam line where we could orient crystals about a mirror plane was at the new SBC 19ID beam line at the Advanced Photon Source (APS) in Argonne. We felt this orientation was necessary to eke out the best anomalous signal from our crystals. But the beam line was not yet open to the public, since it was just being commissioned. Our competitors had already collected data there but it was not clear that we would be able to get time on it. Fortunately, as a result of a request from Peter Moore on our behalf to Paul Sigler, who was on the advisory board for that beam line, we were awarded beam time 2-3 months hence, in late February. It was tragic that only days after he interceded on our behalf, Paul suddenly died of a heart attack.

Given the competition, we wanted to ensure that our data collection at the APS was a success, since it was not clear that we could avoid being scooped if that trip failed. Bil Clemons and Rob Morgan-Warren, a technician who joined us at the LMB, froze over a thousand crystals in the cold room while listening to Johnny Cash on a mini stereo system. We then would take the crystals to Daresbury to screen and group them into crystals that had similar cell dimensions and diffracted well. Not content with this, I sent Bil on a solo trip to Brookhaven where he collected low-resolution data on each of the derivatives to make sure they were bound. He was completely exhausted after having spent 48 hours without sleep.

During our crucial trip to the APS in late February 2000, four of us worked in 12 hour shifts using a large spreadsheet that told us which crystals we had to look at next. Ditlev used his computing skills to streamline our data collection and analysis procedures. We calculated an anomalous difference Fourier map while still at the beam line, and when I saw the large number of strong peaks for our best derivative, much to Rob's amusement, I started dancing around the office saying, "We're going to be famous!"

The maps from the improved data were stunning, and we were on our way to building the structure. With five of us working long hours, were able to build a complete atomic model for the subunit within weeks. Even before we had finished, Andrew Carter had crystallized the subunit with three different antibiotics, and seeing them directly in difference Fourier maps was another great highlight.

The structure of the 30S subunit led to a number of follow-up studies on antibiotics and ligand binding. The most

important of these, largely carried out by James Ogle, led to understanding how the ribosome ensures the accuracy of translation during decoding of the genetic message. Our studies on decoding continue to this day in the context of the whole ribosome.

The Whole Ribosome and Its States

It was always clear that we would need high-resolution structures of the entire ribosome in many states to understand the underlying mechanisms of translation. This problem turned out to be much harder than we had anticipated given the speed with which our 30S work had been accomplished. A stream of dedicated postdocs and students wrestled with the problem for many years. Frank Murphy, assisted by Mike Tarry, worked out procedures for how to make ribosomes that were pure enough to crystallize. Tina Daviter and Ann Kelley introduced methods to purify large quantities of ribosomes. Maria Selmer and Christine Dunham collaborated on a project that led to high-resolution crystals of the whole ribosome with mRNA and tRNA bound, and they were joined by Frank, Sabine Petry and Albert Weixlbaumer to build the structure. This was another frantic effort that had echoes of the 30S race, because we had suddenly heard that Harry Noller, whose lab had solved the whole ribosome at low resolution, now had improved crystals of a ribosome complex and we did not want to be beaten to it after having spent so many years on the problem.

Of the current members of the lab, Martin Schmeing, Caj Neubauer, Rebecca Voorhees, Hong Jin and Yong-Gui Gao work on many different aspects of the translational pathway including elongation, termination and quality control. Two postdocs, Leong Ng and Israel Sanchez have boldly decided to tackle the eukaryotic ribosome, and Alexey Amunts is involved in a joint collaboration with John Walker on the mitochondrial ribosome. Throughout all this, Ann Kelley has become the senior member of the lab. As a longstanding research associate, she is the person who holds the lab together, training new people, making essential reagents, and becoming the general repository of knowledge for the lab. She does this in her usual matter-of-fact way without complaining, and given my other duties in the last few years, the lab would run into great difficulties without her.

Life in Cambridge

Despite our having enjoyed Cambridge thoroughly during my sabbatical year, Vera and I were surprised to find our permanent move there in 1999 difficult. The pay cut and the increase in cost of living were less of a problem because we simplified our lives and never really noticed a difference in our standard of living. But we had left our families, especially our children, behind and there were just the two of us in England.

That isolation has been compensated for by life in Cambridge. Our simple car-free life style, coupled with the rich cultural life of Cambridge and the proximity of London has made our daily lives in Cambridge a pleasure. We have made many good friends who have enriched our lives. At the lab, Kiyoshi Nagai, Daniela Rhodes, Phil Evans, and Andrew Leslie are among many people with whom I have become friends over the years. Every Easter, Vera and I join Phil and his wife Carol, along with Guy and Eleanor Dodson, and Peter and Judith Murray-Rust, to go on a walking holiday. These trips have allowed me to see some of the beautiful countryside of England. We also take bike rides and walks with Alan Coulson (who was Fred Sanger's long time associate and a coauthor on the dideoxy sequencing paper) and his wife Sue, and Ray and Judy Steward. So over the years, we have come to regard England as home.

Fortunately, our family has also survived the transatlantic separation. Tanya is a physician with a master's in public health who works mainly with poor immigrants in Portland, Oregon. Raman, after graduating with a physics degree, obtained a master's degree in music from the Juilliard School and is a cellist with the Daedalus Quartet. My sister Lalita is on the faculty at the University of Washington in Seattle where her work on TB using novel model organisms for both the bacterium and host is allowing her to make important discoveries. My parents lived near her in Seattle, but sadly, my mother, who first encouraged me to go into basic science and later to go to Cambridge, died two years before the Nobel Prize for the ribosome was awarded. My father, who is 84 years old, helped to raise my sister's children and remains physically active and mentally alert, cleaning two houses, cooking and doing the laundry, in addition to trying to establish educational programs in India. Our geographical isolation from our family has been mitigated by the increasing ease of communications, including the use of programs such as Skype.

Reconnecting with India

Since I left India at the age of 19, I had been back only three times in about 28 years. One by-product of my travels was that in early 2002, I was asked to give the first G.N. Ramachandran Memorial Lecture in Chennai. On this trip, I also visited the Indian Institute of Science in Bangalore for a day. This was my first visit to India in 13 years, and my first ever interaction with the Indian scientific community. It started a process by which I not only became more familiar with scientific research at a few institutions in India, but also got to know individual scientists well. In the last few years, I have had a G. N. Ramachandran visiting professorship at the Indian Institute of Science in Bangalore, and have used it to escape the dark and dreary late December–early January period in Cambridge and work in Bangalore on papers and reviews, give lectures and talk to colleagues and especially young scientists there. This reconnection with my Indian roots has given me great satisfaction, and I was touched when in 2008 the Indian National Science Academy elected me as a foreign fellow.

The Politics of Scientific Recognition

People go into science out of curiosity, not to win an award. But scientists are human, and have ambitions. Even the best scientists are often insecure and feel the need for recognition. Our ribosome work led to lots of invitations to give seminars and speak at conferences. It resulted in my election to the Royal Society and the U.S. National Academy of Sciences and also led me to receive a prestigious European prize, the 2007 Louis–Jeantet prize for medicine. Thus in both my scientific efforts and the recognition for it, I had succeeded beyond my wildest dreams.

Although few scientists are foolish enough to enter a field to win a Nobel Prize, ever since the 30S subunit had been solved, people would regularly bring up "the Prize" in conversations whenever I went to conferences or give seminars. It was clear to me that the ribosome was at least as important as other structures that had been awarded the Nobel Prize. But there were many more than three people who had contributed to the ribosome, even if one only counted principal investigators, which itself is a fictional view of the way modern science is done.

While we were solving the structure of the 30S subunit, I had mostly refused to be distracted by going to meetings to speak about our work. So it was something of a shock when only a couple of months after the atomic structures of the subunits came out, a prize in the USA was awarded to just one aspect of the ribosome, peptidyl transferase. It seemed to me that instead of waiting for the impact of the ribosome work to become clear and then thinking hard about what had really made a difference to the field, the committee had hurriedly decided on which three people they wanted to honor and then written a citation around them that would exclude the others. Richard Henderson, my director, suggested that I should accept more invitations to meetings and talks to get our story known if only to get proper recognition for our work, regardless of prizes.

Deep down, I felt that the scientific event that transformed the field more than anything else was the determination of the atomic structures of the ribosomal subunits and the functional studies that followed as a result, to which we had made a major contribution. However, international prizes for work on ribosomes always seemed to go to other people. So over the years, I had gradually come to accept that I would probably not get a major international prize for the ribosome, least of all the Nobel Prize. Once I had accepted that, I felt liberated and happier, but I have to confess that I felt some trepidation each October. Every time I learned the Nobel Prize was for something other than the ribosome, I would be relieved because it was a postponement of what I felt would be the inevitable disappointment. As the years went by, it seemed to me and many other scientists that there would never be a Nobel Prize for the ribosome because the problem of choosing three people out of all the contributors appeared insurmountable.

The Nobel Prize and Its Immediate Aftermath

On October 5, 2009, the Nobel Prize for Physiology and Medicine went for work on telomerase. Since the Chemistry prize had been awarded for biological work the previous year, I was confident that it would not be awarded for the ribosome that year. On the morning of October 7, I was halfway to work when my bicycle developed a flat tire. As a result, I came in quite late and somewhat irritated, and had completely forgotten that it was the day the Chemistry Prize was going to be announced. So when the phone rang soon afterwards and a voice said it was an important call from the Royal Swedish Academy of Sciences, I immediately suspected it was a prank orchestrated by one of my friends like Rick Wobbe or Chris Hill, who like practical jokes. When Gunnar Öquist came on the line and started talking to me, at first I simply refused to believe him and even complimented him on his Swedish accent. Finally, after he was done, I asked if I could speak to one of the committee members, Måns Ehrenberg, whom I knew personally. When I heard his voice, it was with a shock that I realized it was true, a feeling that was reinforced when Anders Liljas and Gunnar von Heijne also came on the line to congratulate me. Two members of my lab, Martin Schmeing and Rebecca Voorhees, had desks just outside the open door to my office and had overheard my end of the entire conversation. They did not share my skepticism and could hardly contain themselves. By the time I got off the phone, they were jumping up and down, and Martin popped open a bottle of champagne he had been saving to celebrate the publication of a paper that had just been accepted in *Science* (Figure 6). In the intervening minutes between the phone call and the public announcement, I was unable to get hold of Vera, because she was taking a walk with Tanya and does not use a mobile phone. It was 2 am in Seattle and 5 am in New York, so I did not want to wake up my father, sister or Raman. Unfortunately, the press was not so considerate.

It was not until I saw the public announcement on the Nobel web site that it fully sank in. Within a few minutes, the phone rang and did not stop ringing for two days. My colleagues at the LMB, many of whom had supported me when I had nothing but an idea, were delighted. They organized the customary drinks celebration in the canteen, for which Mike Fuller bought and served the champagne as he had for all the previous Nobel Prizes awarded to scientists here. After the celebration, Vera and I walked my bicycle home in the rain.

It was touching to get congratulatory messages from old friends and scientific colleagues around the world. I was especially moved by messages from colleagues in the ribosome community including my mentor Peter Moore and Joachim Frank, both great scientists who had made major contributions to the field and were justifiably contenders for the prize themselves. Peter was particularly (and typically) gracious, and seemed proud that his protégé had done so well. Much was made of my prize in India, and I found myself the subject of an entire nation's celebration. I was taken aback by the flood of emails from complete strangers in India, and when they continued unabated for several days, I overreacted to what I felt was an intrusion on my ability to carry out my

Figure 6. Colleagues at the LMB celebrating on October 7, 2009. Top: Aaron Klug, Richard Henderson, Tony Crowther (with me), Kiyoshi Nagai, Daniela Rhodes. Bottom: Phil Evans, Andrew Leslie, Alan Coulson, Vera and I.

work. This angered many people there and a clarification I made only partly mollified them.

The Nobel week in Stockholm in December was surreal and memorable. After Sweden, I went on my usual annual visit to India, but this time with some trepidation because I did not know what the reaction to me would be given the email controversy. I need not have worried, because I was overwhelmed by the warmth and affection from both members of the public and my scientific colleagues there. I was honored that the Government of India decided to bestow upon me their second highest civilian award, the Padma Vibhushan. I have come to realize that I have inadvertently become a source of inspiration and hope for people in India simply by the fact that I grew up there and went to my local university, but could nevertheless go on to do well internationally.

On my return to Cambridge in early January, things slowly began returning to normal after the euphoria of the autumn. I began to realize that the Nobel Prize could be seen not just as an affirmation of my past work but also as an encouragement to continue to work on interesting problems. Certainly, it seems to have fired up people in my laboratory, and I look forward to the struggles ahead as we try to answer some of the hard questions in our field and beyond. Looking back on my life so far, I feel a deep sense of gratitude for having been able to lead such a rich life both intellectually and personally.

Nobel Lecture

Introduction

During the decade following the discovery of the double-helical structure of DNA, the problem of translation, namely how genetic information is used to synthesize proteins, was a central topic in molecular biology. A crucial idea, proposed by Crick,[1] was that a small intermediary molecule containing a trinucleotide that base-paired with the genetic template would bring along a covalently linked amino acid for addition to the polypeptide chain. This idea, called the "adapter hypothesis", was soon confirmed when a "soluble" or "s" RNA was discovered that had covalently linked amino acids.[2,3] It is sad to note that two of the key scientists involved in this work, Hoagland and Zamecnik, both died in the fall of 2009 around the time when the Nobel Prize for the ribosome was announced. The sRNA, renamed tRNA, is a central player in the process of translation. Unlike the trinucleotide originally envisioned, it actually consists of about 76 nucleotides,[4] with the anticodon end that recognizes the triplet codon on mRNA about 75 Å away from the 3′ end that has the covalently linked amino acid.[5,6]

At the same time, scientists studying the ultrastructure of the cell noticed that newly synthesized proteins were localized in particles on the endoplasmic reticulum,[7] suggesting that a large complex assembly was involved in the process of translation. These particles could be isolated and were originally called the "ribonucleoprotein particles of the microsomal fraction." Fortunately, at an early meeting, Howard Dintzis proposed that the particle be called the "ribosome" and the name has been used ever since.[8]

Decades of subsequent work established a number of salient facts such as the shape of ribosomes, their composition, the number of tRNA binding sites, the main roles of the two subunits and the involvement of protein factors, many of them GTPases, at specific stages of translation.[9] Ribosomes from all species consist of two subunits that dissociate reversibly.[10,11] The subunits of the 70S bacterial ribosome are designated 30S and 50S, whereas those of the 80S eukaryotic ribosome are designated 40S and 60S after their respective sedimentation coefficients. The small subunit binds mRNA and the anticodon stem loops of tRNA. The large subunit contains the peptidyl transferase center where catalysis of the

peptide bond formation occurs. In both bacteria and eukaryotes, the ribosome is about two-thirds RNA and one-third protein by mass, although in mammalian mitochondria, this ratio is reversed.[12]

I began studying ribosomes as a postdoctoral fellow in Peter Moore's laboratory in 1978. There I participated in a project to determine the spatial location of the 30S proteins by neutron scattering, which culminated in the so-called "neutron map".[13] In 1983, I joined the Biology Department at Brookhaven National Laboratory as a staff scientist, and just two years later, Stephen White, who had moved from Wittmann's department at the Max Planck Institute for Molecular Genetics in Berlin joined me as a colleague. He had brought with him several crystallization projects of individual ribosomal proteins. I decided to use the then new more highly regulated version of the T7 expression system,[14] which allowed us to clone the genes for and overexpress many of these proteins.[15] We used this system to make large quantities of almost any desired ribosomal protein, and to label them with cysteines (for mercury derivatives) or with selenomethionine. The result was that after a long hiatus, the pace of determination of structures of ribosomal proteins increased quickly.[16] However, it gradually became clear that these structures by themselves would not be ultimately useful in understanding ribosome function, even when used as labeling markers for footprinting studies or in modeling into electron microscopy (EM) maps of the ribosome. Only the high-resolution structures of entire ribosomal subunits or the whole ribosome would suffice.

The first indication that ribosomes were identical molecules that could be packed into a crystalline lattice came from the observation of naturally occurring two-dimensional crystalline arrays.[17] However, a major breakthrough was achieved when Yonath, Wittmann and their co-workers produced the first three-dimensional crystals of the 50S subunit from *Bacillus stearothermophilus*.[18] After nearly a decade of work and the exploration of other species, in another landmark, Yonath and co-workers obtained crystals of the 50S from *Haloarcula marismortuii* that diffracted to 3 Å resolution,[19] suggesting that an atomic structure of the subunit would be possible if the crystallographic difficulties could be overcome. Another major contribution was made by the group of Marina Garber in the Institute for Protein Research in Puschino, Russia. They reported the first single crystals of both the 30S subunit and the 70S ribosome from *Thermus thermophilus*.[20,21] Similar crystals of the 30S subunit from the same species were subsequently obtained by Yonath and co-workers.[22] Finally, in a crucial technical advance, Hope had shown that data collection at 100 K minimized radiation damage for several proteins,[23] and he and Yonath collaborated to show that this method allowed data collection from crystals of ribosomal subunits.[24]

Determining the Structure of the 30S Subunit

Thus in 1995, when I moved to the University of Utah, well-diffracting crystals of the 50S subunit had existed for some time, but with limited progress on determining its structure. However, there were no crystals of the 30S subunit that diffracted to high resolution. Given my longstanding interest in ribosomes and in the 30S subunit in particular, I therefore decided to try and obtain good crystals of it with the goal of determining its structure. At about this time, Joachim Frank and co-workers had helped to develop the use of single particle reconstruction methods in conjunction with cryoelectron microscopy (cryoEM). In their studies, they found that the conformation of the 30S subunit was different in inactive, active, and 50S-bound states.[25] This suggested that the difficulty in obtaining well-diffracting crystals might be due to the conformational variability of the 30S subunit. We had recently solved the isolated structure of initiation factor IF3, which was thought to bind one of the regions of the 30S subunit that was conformationally variable.[26] Accordingly, we started with the idea that the binding of IF3 to the 30S subunit might fix the latter in a single conformation suitable for producing better crystals. We began by initiating a collaboration with Joachim Frank and Raj Aggrawal to determine a cryoEM structure of the complex of IF3 with the 30S subunit.[27] Although later studies showed that our interpretation of the density in terms of the domains of IF3 was only partly correct,[28,29] the difference density nevertheless clearly showed that IF3 bound to the conformationally variable platform region of the 30S subunit. We therefore set about trying to crystallize the 30S subunit in complex with IF3. It is just as well that we did not need a complex with IF3 to determine the structure of the 30S subunit or we would still be waiting.

To begin with, we decided to reproduce the low-resolution crystals from *Thermus thermophilus* obtained earlier by the Puschino group[20,21] because that would tell us whether our ribosome preparations were good enough to crystallize. Marina Garber, who led the group that crystallized the 30S subunit and 70S ribosomes in Puschino, told us that the use of hydrophobic chromatography was useful to get ribosomes that were good enough to produce 70S crystals. I had some previous experience in the use of this method to make ribosomes,[30] so with the help of Bob Dutnall, a postdoc working on histone acetyltransferases, I tested a number of different hydrophobic resins before settling on the Poros-ET column made by Biocad.[31]

We obtained initial crystals of the 30S subunit within a few months. These crystals lacked ribosomal protein S1 (Figure 7A), which is present in variable stoichiometry in preparations of the 30S subunit. Although we could get large crystals even from this heterogeneous preparation, the reproducibility and size improved significantly when we systematically removed S1 quantitatively prior to crystallization (Figure 7B), and these crystals diffracted to beyond 3 Å resolution (Figure 7C).[31,32] Thus we had in hand crystals that were in principle capable of yielding an atomic structure of the 30S subunit provided we could collect complete data to the resolution limit and solve the phase problem.

When we began, the difficulties of obtaining good phases for ribosome-sized molecules had been apparent for some time, and it was clear that the field could use fresh ideas. Only a few years earlier, I had used the technique of multiwavelength anomalous diffraction (MAD) pioneered by

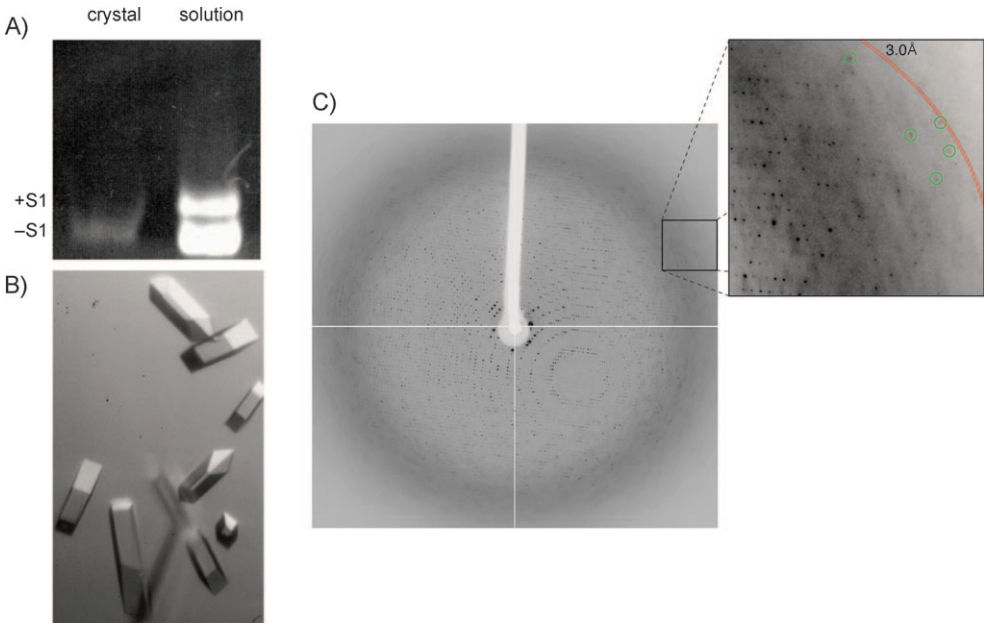

Figure 7. A) An agarose–acrylamide non-denaturing gel of redissolved crystal of the 30S ribosomal subunit (showing they lack protein S1) and the input sample that contained a mixture of 30S subunits with and without protein S1. B) Crystals of 30S subunits. C) Diffraction from the crystals in (B), showing spots to about 3 Å resolution.

Wayne Hendrickson and Keith Hodgson[33,34] in conjunction with the use of incorporated selenomethionine as an anomalous scatterer[35] to solve the structure of the globular domain of histone GH5.[36] In the process of solving that structure, following an initial suggestion by Eleanor Dodson, I had shown that the treatment of MAD data as a special case of multiple isomorphous replacement with anomalous scattering produced excellent maps, which was surprising considering the very low anomalous and dispersive signal of a few electrons from a selenium atom.[36,37] The reason for this is that although both the anomalous and dispersive signals from selenium are only a few electrons rather than the around 80 electrons of a "heavy" atom like gold or mercury, there is far less noise from scaling or nonisomorphism in MAD, so that the signal to noise is actually much better than that of a typical solution using isomorphous replacement.

This naturally led me to ask what it would take to have sufficient anomalous scattering to produce a measurable signal from crystals of a 30S subunit. A back of the envelope calculation in conjunction with Ethan Merritt's web site that calculates the expected signal (http://skuld.bmsc.washington.edu/scatter/AS_signal.html) showed that there were too few methionines in the 30S subunit to provide a measurable signal. At the IUCr meeting in Seattle in 1996, Wayne Hendrickson described the enormous signals from the M4 or M5 edges of uranium,[38] whose anomalous scattering could be as much as 100 electrons. However, a conversation with Craig Ogata, who had carried out these experiments, made me realize that the technical difficulties of carrying out such an experiment would be enormous because of the wavelengths required and the consequent radiation damage. A reasonable compromise was the use of the LIII edges of lanthanides that had produced spectacular maps.[39] A calculation showed that as few as 10–20 well-bound lanthanide sites would yield a sufficiently strong anomalous signal to derive phase information from the 30S subunit. A paper on the cleavage of ribosomal RNA by lanthanides in alkaline pH suggested that there were indeed a number of well defined sites for lanthanides in the 30S subunit.[40] We therefore decided to embark on trying to phase the 30S subunit using anomalous scattering from the LIII edges of lanthanides. I believed that we would probably be able to locate the anomalous scatterers using direct methods, and tests done after the fact suggested that indeed this would have been the case.

As we embarked on this, two developments occurred that influenced our course of action. The first, in 1996 was the publication of the P4–P6 domain of the group I intron[41] which was solved using MAD from an LIII anomalous edge. The compound that contributed most to phasing of this large RNA was osmium hexamine. It was clear from the characteristics of its binding site[42] that this compound would probably bind to many more sites in the 30S subunit than most lanthanides. However, it was not commercially available, and since Henry Taube, who had provided the compound to the authors of the P4–P6 study, no longer had any of it to spare, I asked my friend and former colleague at Brookhaven, Bruce Brunschwig (now at Caltech), if he could make it for me. He very kindly agreed and with his associate Mei Chou made enough for our studies.

The other development was the publication of the 9 Å structure of the 50S subunit from Yale,[43] which for the first time showed right-handed helices for double-stranded RNA, which was a major breakthrough. To obtain this, they had used heavy-atom tungsten clusters, previously suggested by Yonath and co-workers.[44] However, they had also obtained starting phases from molecular replacement using a cryoEM reconstruction of the 50S subunit done by Joachim Frank and his colleagues as a starting model. The W18 cluster was directly visible in difference Patterson maps, so it is not clear that molecular replacement phases from a cryoEM model was actually required, but they were able to confirm the location of the W18 clusters using difference Fourier maps from those phases. Accordingly, we decided to try both molecular replacement using cryoEM and use heavy-atom clusters as a way of obtaining starting phases from which we could locate our anomalous scatterers at LIII edges by difference Fourier methods. We obtained a set of tungsten clusters ranging from 11 to 26 atoms from Michael Pope of Georgetown University,

who generously sent them to us, as he had previously to Yonath and Steitz. A different cluster, tantalum bromide, was kindly provided by Gunther Schneider of Stockholm University and from Jan Löwe, whom I joined as a colleague at the MRC Laboratory of Molecular Biology in Cambridge soon after collecting the data described in the following paragraph.

Attempts at molecular replacement using cryoEM reconstructions of the 30S subunit at ca. 25 Å resolution provided by Joachim Frank as a starting model did not yield a solution in our hands. However, during a single trip to the NSLS at Brookhaven, we collected data at the peak of the fluorescence spectrum at the LIII edges for the various tungsten clusters, tantalum bromide, and lanthanides as well as osmium hexamine. The peak corresponds to the maximum of f'' (Figure 8A), resulting in maximizing anomalous differences between Bijvoet pairs. Even while at the beamline, we could see clear anomalous difference Patterson peaks for the W17 cluster (Figure 8B). We accordingly collected two more wavelengths on this derivative to maximize the dispersive signal by collecting at the "rising edge" and "falling edge" of the fluorescence spectrum. These very close wavelengths correspond to the minimum and local maximum of the real part f' of the anomalous scattering factor, and by choosing them, we maximized the dispersive or "isomorphous" difference in f' between the two wavelengths, which resulted in strong peaks for the difference Patterson map at the same positions as those for the anomalous difference (Figure 8C). Using both the dispersive and anomalous differences in MAD phasing yielded us initial phases to about 9 Å resolution. We used these phases to locate all of the other derivatives. Because the various derivatives were not isomorphous with one another, we grouped together those derivatives that were isomorphous to medium resolution to solve the structure of the 30S to 5.5 Å resolution.[32]

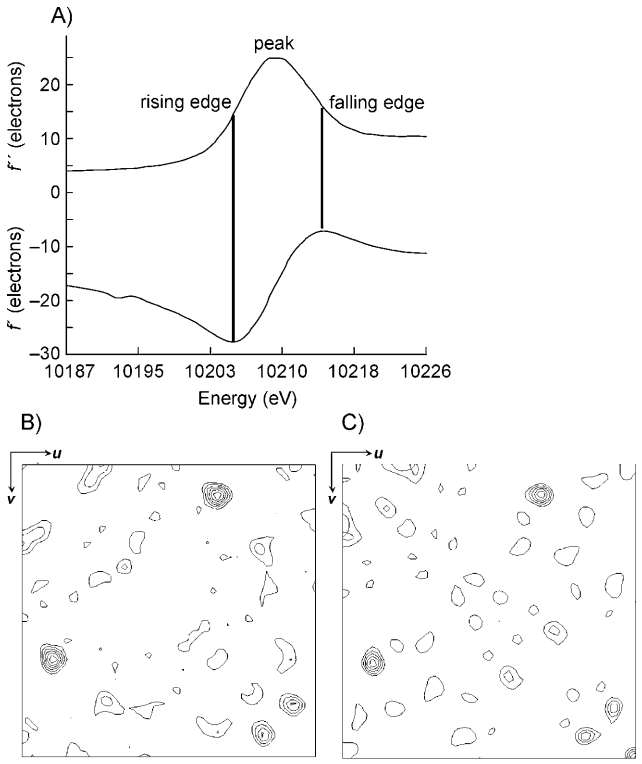

Figure 8. A) Spectrum of the LIII edge of tungsten, courtesy of S. Myers and D. Schneider, Brookhaven National Laboratory. Data were initially collected at the peak of f'' where the anomalous differences are maximized, and subsequently at the two wavelengths that correspond to the minimum and local maximum of f'. B) Harker section of anomalous difference Patterson map of a W_{17} derivative of the 30S crystals, generated from the data at the peak of f'' showing peaks corresponding to the W_{17} clusters in the structure. C) Harker section of Patterson maps of the isomorphous (dispersive) difference between the data collected at the minimum and local maximum of f', showing peaks at identical locations as in (B).

This structure represented a breakthrough in several respects. It showed that reliable phases could be obtained with our 30S subunit crystals. We could clearly see double-helical density for RNA and recognize the long penultimate helix 44 of 16S RNA at the interface (Figure 9A). Because the resolution was sufficiently high to recognize individual proteins of known structure (Figure 9B), we were able to place all of the 30S proteins whose structure was previously known, as well as protein S20, which was a three-helical bundle at the "bottom" of the subunit. Finally, in conjunction with biochemical data, especially footprinting data from Noller and co-workers,[45,46] we were able to identify stretches of RNA adjacent to specific proteins and thereby trace the fold of most of the central domain, resulting in a partial model for the 30S subunit (Figure 9C). Since the crystals diffracted to ca. 3 Å, the way to an eventual atomic structure seemed clear.

Unfortunately, the progress to high resolution was beset by several problems. Because the diffraction at high resolution was quite weak, it became necessary to take much longer exposures in order to obtain sufficiently accurate data to the resolution limit. However, the crystals suffered from sensitivity to radiation damage at these higher exposures, requiring multiple crystals for a complete data set. At the same time, they showed variability in cell dimensions resulting in non-isomorphism which made it impossible to scale data together from multiple crystals. These two mutually incompatible problems made it difficult for us to make progress for several months. The problem was solved partly by brute force in which we screened hundreds of crystals and grouped them into sets that had similar cell dimensions. We also noticed that crystals soaked in osmium hexamine seemed to have consistent cell dimensions, and decided to soak all crystals in the isostructural cobalt hexamine, which greatly reduced crystal-to-crystal non-isomorphism. The structure was solved using mainly anomalous scattering from a variety of lanthanides and osmium hexamine at their respective LIII edges. In hindsight, we found that anomalous scattering from just the osmium hexamine derivative in conjunction with density modification would have given us equally good phases. Given his prior work on the P4–P6 domain, it is not surprising that Cate independently adopted an essentially identical strategy of anomalous scattering from osmium or iridium hexamine for the solution of the 5.5 Å structure of the 70S ribosome in the Noller laboratory,[47,48] and it was also essential for solving the 50S subunit to high resolution.[49]

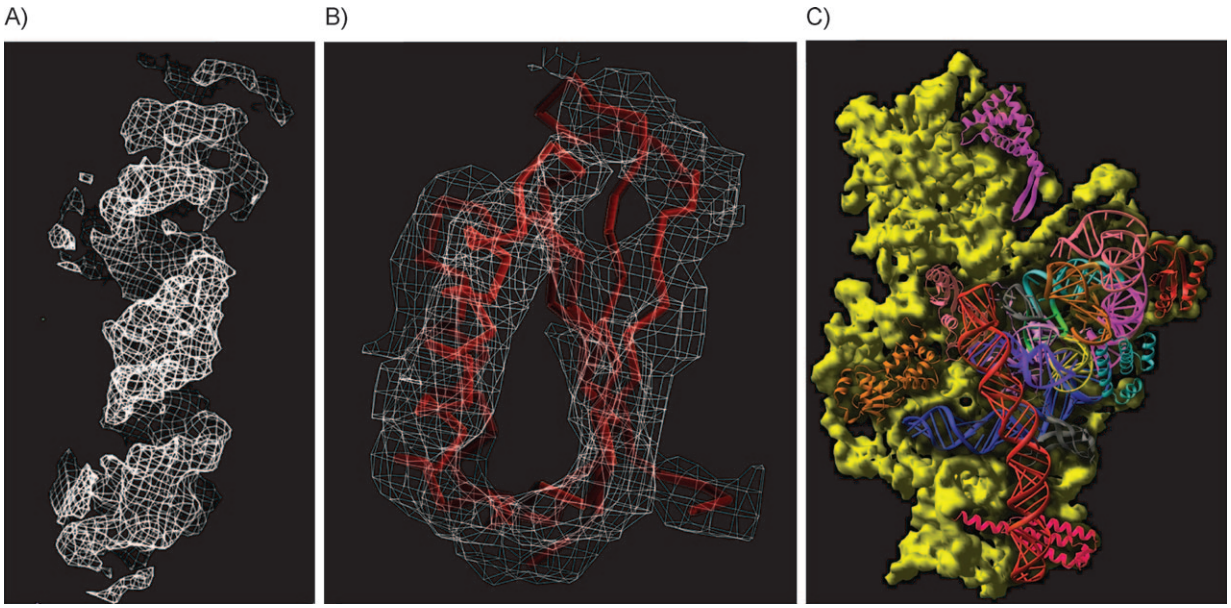

Figure 9. The 5.5 Å structure of the 30S subunit. A) Electron density for right-handed double helical RNA showing the two strands and bumps for the phosphate groups. B) Electron density for ribosomal protein S6, showing the fit of the isolated protein structure.[103] C) A partial model of the 30S subunit in which the fold of the central domain has been traced, and all proteins of previously known structure as well as S20 had been placed in the electron density. Reproduced from Ref. [32].

The Structure of the 30S Subunit

We finally succeded in solving the atomic structure of the 30S subunit from *Thermus thermophilus* and an essentially complete atomic model that included all the RNA and proteins present was refined to 3 Å resolution[50] (Figure 10). At about the same time, the Yonath group, using slightly different methods and using crystals that required the binding of heavy-atom clusters to diffract well, independently solved a structure of the 30S subunit to 3.3 Å.[51] This structure, besides

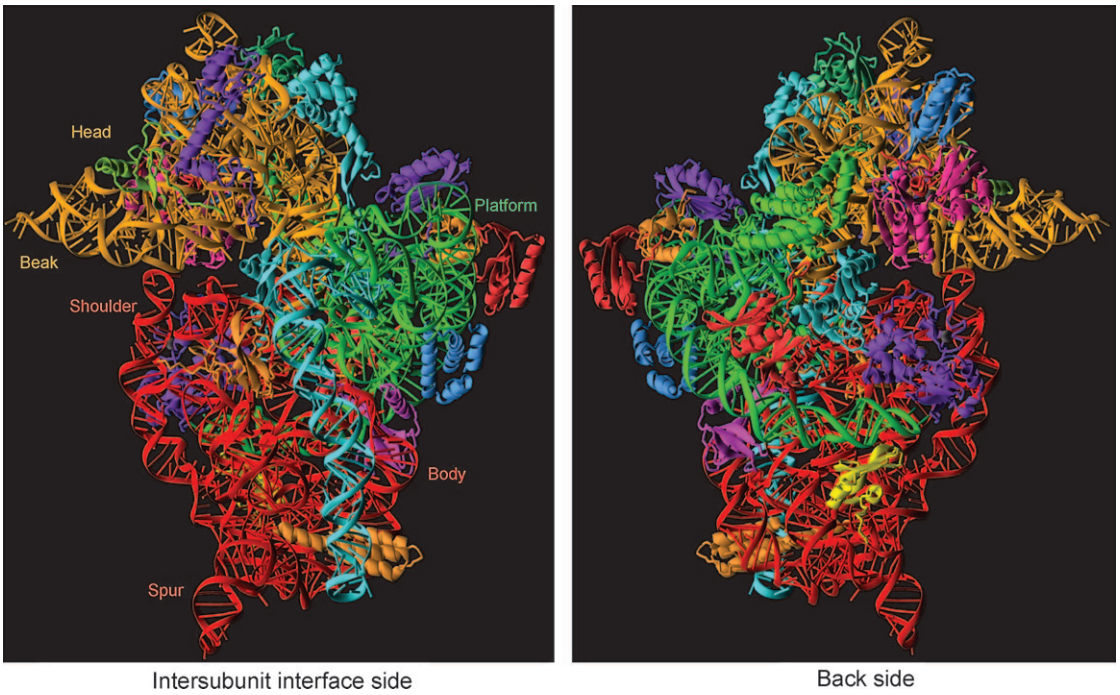

Figure 10. The atomic structure of the 30S subunit refined to 3 Å resolution. A) The "front" or intersubunit interface side which is relatively free of proteins, with the various features of the subunit 30S labeled by their usual name. B) The "back" or solvent side, which is covered extensively by ribosomal proteins.

being less complete, had a number of discrepancies with our structure,[52] but a subsequent structure published by that group the following year[53] at 3.2 Å agreed well with our original structure.

The structure of the 30S subunit provided a firm basis for the interpretation of decades of biochemical and genetic data.[50,52,54] The superposition of the mRNA and tRNA located in a 7.8 Å structure of the whole ribosome,[47] allowed us to analyze the interactions of these ligands with the 30S subunit. We were able to do this in nearly atomic detail for the P site, for a fortuitous reason: A particular feature of this crystal form was that a stem loop of 16S RNA (the "spur"; see Figure 10) of a neighboring molecule was inserted into the P site of the 30S subunit, where it mimicked the anticodon stem loop of P-site tRNA and interacts with the 3′ end of 16S RNA which had folded back into the P site, in an analogous manner to how the tRNA anticodon interacts with the mRNA codon.[54]

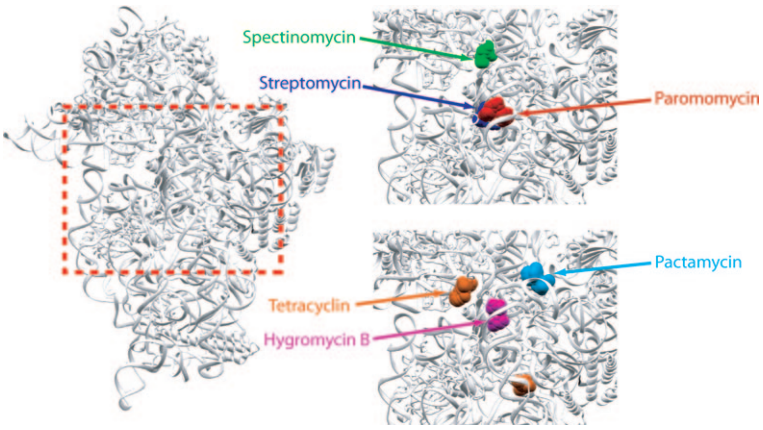

Figure 11. Antibiotics bound to the 30S subunit. The box on the left is the region of the 30S subunit where the mRNA and tRNA ligands bind, and as can be seen in the expansions of the box on the right, it is also where key antibiotics bind to key sites and cause loss of ribosome function in various ways.

In addition to details of the ligand binding sites, the structure revealed some very general principles. The functional sites that bound RNA ligands and the subunit interface side that formed contacts with the 50S subunit consisted almost entirely of RNA, with the exception of protein S12 near the decoding center. The active sites were highly conserved, including tertiary interactions. Many of the proteins had long extended tails at the N- or C-termini that snaked through 16S RNA and made intimate contacts with it. These features were also observed in the 50S subunit.[49,55] The protein–RNA interactions in the 30S subunit have been analyzed in detail.[52]

Use of the 30S Structure for the Study of Antibiotic Binding

With a refined structure of the 30S subunit, it became straightforward directly to locate several antibiotics bound to the 30S subunit from difference Fourier maps using data collected on antibiotic complexes. Indeed, back-to-back with the 30S structure itself, we also published the structure of its complex with three different antibiotics bound simultaneously: streptomycin, spectinomycin, and paromomycin.[54] These were followed soon afterwards by three more, tetracycline, pactamycin, and hygromycin B[56] (Figure 11). These represented the first reports of the detailed structure of antibiotics bound to entire ribosomal subunits, although the structure of paromomycin bound to a fragment of 16S RNA containing its target had been determined previously by NMR spectroscopy.[57] Each of these antibiotic structures yielded insights into their respective mechanisms of action. Moreover the detailed structure of antibiotics bound to ribosomal subunits is leading to the development of new and more effective antibiotics that may prove useful against resistant strains of pathogenic bacteria. One antibiotic, paromomycin, has also led to useful insights into decoding as discussed below.

The Problem of Decoding of the Genetic Message

The accuracy of translation depends on several independent processes that individually need to be at least as accurate as the overall accuracy. These processes include the recognition of the appropriate tRNA by the aminoacyl synthetase that charges it with the correct amino acid, and maintenance of the mRNA reading frame during translation. An equally important process is decoding, which is the codon-dependent selection of the appropriate aminoacyl tRNA during protein synthesis and involves base pairing of the codon on mRNA with the anticodon on the tRNA.[58] The structure of the 30S subunit and later the entire ribosome have shed much light on decoding.

Ever since the discovery of the genetic code, it has been known that the code is highly degenerate: many codons that differ at the third position code for the same amino acid. The fact that there are fewer tRNAs than there are codons led to the wobble hypothesis in which certain kinds of mismatches are tolerated at the third or "wobble" position, whereas strict Watson–Crick pairing is required at the first two positions of the codon.[59]

The free-energy difference due to a mismatch in base pairing is not sufficient to account for the accuracy of translation. The earliest evidence that the ribosome was involved in the accuracy of tRNA selection came when the antibiotic streptomycin, which binds to the 30S subunit, was found to increase the error rate of protein synthesis.[60] This led to the proposal that the 30S subunit had a decoding center in which it "inspects" the pairing of a codon with an anticodon in much the same way that an enzyme senses the precise pairing of its substrate. However, this view of direct inspection ran into difficulties when a fidelity mutation, the Hirsh suppressor, was discovered at a location far from the tRNA anticodon,[61] which led to the following alternative view.

Aminoacyl tRNA is initially brought into the ribosome as a complex with EF-Tu and GTP. In a view of decoding, termed kinetic proofreading,[62,63] incorrect tRNAs can dissociate before or after GTP hydrolysis by EF-Tu. The overall

selectivity can thus be as high as the product of both selection steps because they are separated by the essentially irreversible step of GTP hydrolysis. Experimental evidence for proofreading came when it was shown that the incorporation of an amino acid by a near-cognate tRNA (which contain a single subtle mismatch between codon and anticodon) require more the hydrolysis of many more GTPs than the cognate case.[64,65] In this view, both the ribosome and tRNA were passive participants in translation, with mutations altering accuracy by modulating the rate at which GTP was hydrolyzed by EF-Tu. In principle, this could explain how mutations distant from the codon–anticodon pairing could affect accuracy.

This view has required significant revision. Careful studies on the stability of RNA helices show that the free-energy differences from a base-pairing mismatch can account for a factor of 5–10 in selectivity[66] rather than the factor of 100 assumed previously. Therefore, even with proofreading, the overall accuracy of the ribosome could not be accounted for by base-pairing alone. Pre-steady state kinetic experiments that dissected the various steps in tRNA selection showed that the *forward* rates of GTPase activation and accommodation (movement of tRNA into the peptidyl transferase center) were dramatically higher for cognate compared to near-cognate tRNA.[67] This suggested that cognate tRNA more efficiently induced conformational changes in the ribosome into a productive form that accelerated GTPase activation or accommodation, consistent with earlier suggestions from NMR studies on a portion of the decoding site.[68] Several aminoglycoside antibiotics bind to the decoding center and increase the error rate of translation. Kinetic studies showed that a main effect of one such antibiotic, paromomycin, was less to increase the affinity of incorrect near-cognate tRNAs but rather to allow such tRNAs to accelerate GTPase activation.[69] Presumably, paromomycin induced conformational changes similar to those induced by cognate tRNAs even without the antibiotic. Subsequent equilibrium binding studies also showed that the increase in stabilization of near-cognate tRNAs due to paromomycin was minimal.[70]

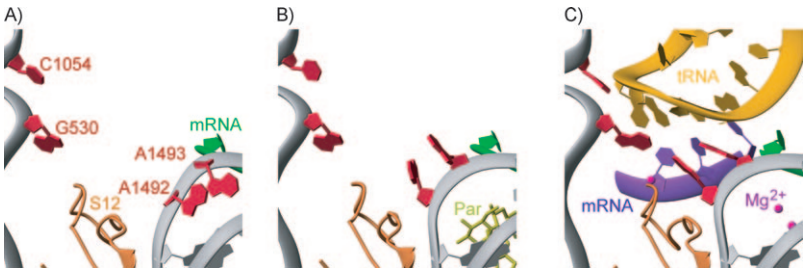

Figure 12. The decoding center of the ribosome where the mRNA codon and tRNA anticodon interact. A) The decoding center in the empty 30S subunit. B) The binding of the antibiotic paromomycin induces a conformational change in the bases A1492 and A1493, so that they are in a position to interact with the codon–anticodon base pairs. C) The base pairing between the anticodon of tRNA and the codon induces a change not only in A1492 and A1493 but also in G530 so all three bases interact with the minor groove of the codon–anticodon minihelix. Adapted from Ref. [72].

Insights into Decoding from Structures of the Ribosome

Possibly one of the most interesting functional insights from the structure of the 30S subunit has come from studies on codon–anticodon interactions at its decoding center. The structure has allowed a molecular explanation for the biochemical observations described above. Importantly, the structure of the 30S subunit bound to paromomycin showed that the antibiotic stabilized the conformation of two key bases, A1492 and A1493, in an orientation which suggested that they would be able to directly inspect the minor groove of the codon–anticodon helix[54] (Figure 12 A and B).

Testing the idea that ribosomal bases directly and specifically inspect codon–anticodon base pairing seemed difficult, because crystals would not grow in the presence of mRNA. However we noticed that the A site of the 30S subunit where decoding occurs seemed to be exposed to large solvent channels in the crystal, suggesting that small macromolecules that bound to the A site could be soaked directly into the crystals. We tested this idea initially by soaking in the small protein initiation factor IF1, which was known to bind to the A site. We could see electron density for the factor in difference Fourier maps and thereby determine the structure of its complex with the 30S subunit.[71]

Once we knew that the A site was accessible to large ligands in the crystal, we could begin structural studies on decoding by soaking RNA oligonucleotides that mimicked the A-site mRNA codon and the tRNA anticodon stem–loop (ASL) into crystals of the 30S subunit. The data showed that the binding of cognate tRNA to the 30S subunit induced a change in the conformation not only of A1492 and A1493 but also of G530, which is part of the "530 pseudoknot" of 16S RNA[72] (Figure 12 C). These three universally conserved bases lined the minor groove of the codon–anticodon helix in such a way that the geometry of the base pair was monitored at the first two positions but not at the wobble position (Figure 13). The structure provided a rationale for the wobble hypothesis[59] and the degeneracy of the code, in which strict Watson–Crick complementarity between the codon and anticodon is required at the first two positions but not the third.

The binding of cognate tRNA also induced global conformational changes in the 30S subunit, in particular a movement of the shoulder of the 30S subunit relative to the rest of the body.[70,72] This conformational change was not observed with near-cognate tRNA unless the antibiotic paromomycin was also present. This suggested that the conformational change induced by cognate tRNA could also be induced by near-cognate tRNA in the presence of paromomycin. Together, these structures complemented kinetic data that suggested that cognate but not near-cognate tRNA induced a conformational change that enhanced the

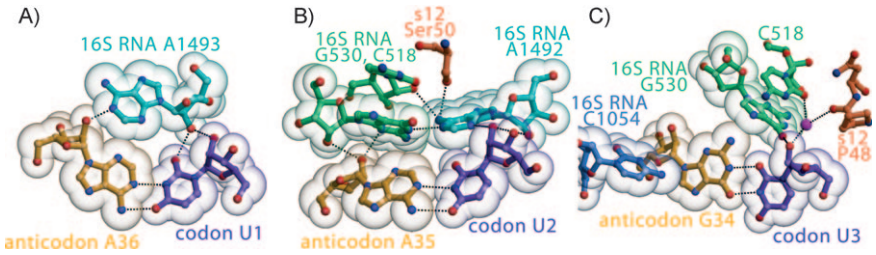

Figure 13. Interaction of conserved ribosomal bases with the three codon–anticodon base pairs showing a specific recognition of Watson–Crick geometry at the first two positions (A and B) and a tolerance of a GU wobble pair at the third position (C). Adapted from Ref. [72].

rate of GTP hydrolysis by EF-Tu,[67] while paromomycin enhanced the rate of GTP hydrolysis even for near-cognate tRNA.[69]

A further examination of the conformational change showed that many mutations in the 30S that affected accuracy were at or near an interface between two domains of the subunit (Figure 14). These two domains moved relative to one another upon tRNA binding in a transition from an open to a closed form of the 30S subunit. Mutations or antibiotics that made this domain closure easier lowered the accuracy of tRNA selection and those that made it more difficult to reach the closed form increased the accuracy of tRNA selection.[70] The conformational changes during decoding allowed the rationalization and integration of disparate genetic and biochemical data in terms of a common mechanism.[58,70]

The work on the 30S subunit suggested that the domain closure from an open to a closed form was required for GTPase activation and thereby tRNA selection.[70] Why should this be the case? The answer was suggested by

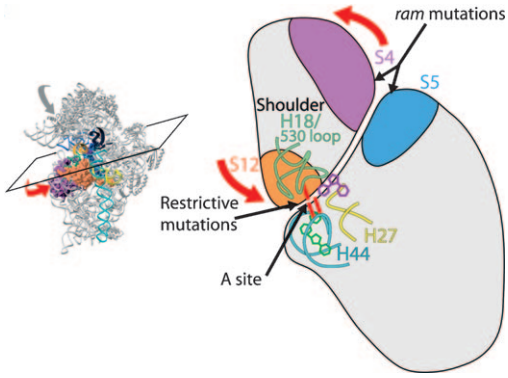

Figure 14. How conformational changes and a domain closure in the 30S ribosomal subunit can explain the effect of antibiotics or mutations on accuracy. On the right is a schematic cross-section of the 30S subunit (as shown by the plane cutting the 30S subunit on the left), in the region of the decoding center and proteins S4 (violet), S5 (blue), and S12 (orange). G530 and A1492/3 are represented by red bars; helices H44, H27, and H18 (with the G530-loop) are cyan, yellow, and turquoise, respectively. The rotation of the shoulder domain (red arrows) during the transition to the closed 30S conformation disrupts an interface between S4 and S5, while the H18/530–loop/S12 region forms new contacts to H27 and H44. Mutations in these regions either increase or decrease mRNA misreading. Paromomycin (dark green rings) and streptomycin (dark pink rings) induce translational errors by facilitating domain closure. Adapted from Ref. [72].

considering domain closure in the context of cryoEM structures of the complex of EF-Tu and tRNA bound to the ribosome.[73] The domain closure, in particular the movement of the shoulder of the 30S subunit, would bring the 30S closer to the EF-Tu and tRNA. We suggested that the domain closure would thereby help trigger GTP hydrolysis by EF-Tu, leading to its release and accommodation of the tRNA in the A site of the peptidyl transferase center. Subsequent cryoEM studies showed that the tRNA in complex with EF-Tu was distorted when bound to the ribosome.[74,75] This led us to suggest a model for decoding in which the additional binding energy from the minor-groove recognition of codon–anticodon base pairing is used to induce a domain closure in the 30S subunit. This domain closure would in turn stabilize the transition state needed for GTP hydrolysis by EF-Tu, which is characterized by a distorted tRNA.[70] Such a model could rationalize not only mutations in the ribosome but also those in the tRNA that affected fidelity. After GTP hydrolysis, the strong interactions at the decoding center would hold the anticodon loop in place while the release of EF-Tu would allow the highly distorted tRNA to relax into the peptidyl transferase center.[70] The idea of a distorted tRNA relaxing into the peptidyl transferase center was also characterized as a "molecular spring",[76] and echoes earlier ideas by Yarus and co-workers that the dynamics of tRNA were important for fidelity.[77]

Although the resolution of cryoEM has been steadily improving, producing structures of the 70S ribosome in complex with EF-Tu at previously unimaginable resolutions of 6–7 Å,[78,79] it was clear that molecular details of the interactions of EF-Tu and aminoacyl tRNA with the ribosome, and thus a further understanding of decoding, would require a higher-resolution structure. However, achieving this was slow for the reasons outlined below.

A High-Resolution Structure of the 70S Ribosome

The crystallization of the 70S ribosome that began in the mid-1980's in Puschino[20] eventually culminated in the structure of the entire 70S ribosome with mRNA and tRNA at 5.5 Å resolution.[48] This work represented a major step forward because it was the first molecular interpretation of the intersubunit interface and the interactions of the mRNA and tRNA ligands with the ribosome. However, at that resolution, it relied on the atomic structures of the 30S and 50S subunits solved previously[49,50] to interpret the electron density in molecular terms. Indeed, those regions interpreted ab initio such as the L1 or L7/L12 stalks that were disordered in the 50S subunit structure proved to be incompatible with subsequent high-resolution structures of those components.[80,81]

A second major landmark was the crystallization and structure determination of the *E. coli* ribosome at 3.5 Å

resolution.[82] This was the first 70S ribosome structure at a resolution high enough for proper refinement of an atomic model, and among other things, resulted in a clarification of the nature of the intersubunit bridges. Moreover, because *E. coli* was the standard organism for the biochemical and genetic study of the ribosome, it was an important reference structure. Nevertheless, the crystal form was incompatible with the binding of full-length tRNAs, limiting its usefulness for functional studies. Thus it was clear that the field still needed new crystal forms of the ribosome that diffracted to high resolution in various functional states of translation.

We thus embarked on an effort to identify completely new crystal forms that would diffract well. To purify ribosomes, we again used hydrophobic chromatography as we had for the 30S subunit, but this time we used Toyopearl butyl-650S, which was suggested by Marina Garber. We found that the profile was temperature sensitive, and at 4 °C but not at room temperature, it was possible to separate 70S ribosomes from excess 50S subunits as well as from a fraction that contained an active ribonuclease that co-purified with ribosomes. Ribosomes using our protocol also lacked any endogenously bound tRNA that might lead to heterogeneity, and were so pure that the individual charge states of whole ribosomes could be resolved by mass spectrometry.[83] This led to an initial breakthrough in which we obtained a tetragonal form of the 70S ribosome, which yielded crystal structures to about 6 Å resolution of the release factors RF1 and RF2 bound to their respective stop codons on mRNA.[84] While these structures were a step forward, they were ultimately unsatisfying because the resolution prevented us from obtaining definitive details of the interactions of the factors with the stop codon or with the peptidyl transferase center.

In the course of trying to crystallize a complex of elongation factor G (EF-G) trapped on the ribosome by fusidic acid in the post-translocational state after GTP hydrolysis, we obtained a new orthorhombic crystal form of the ribosome that initially diffracted to about 4.5 Å resolution. However, an initial electron density map showed no evidence for EF-G in the structure, and indeed, bound EF-G would clash with a neighboring ribosome in the crystal lattice. Nevertheless, we felt this new form was sufficiently promising to continue improving, and eventually obtained a structure of the whole ribosome with mRNA and tRNA ligands at the surprisingly high resolution of 2.8 Å (Figure 15).[85] This structure provided a wealth of information about the interaction of the mRNA and tRNA ligands with the ribosome, the nature of the intersubunit interface and the role of magnesium ions in the structure. But perhaps as important as the structure itself was the fact that this new crystal form provided a route to determine the structure of the ribosome in complex with various factors. We initially used it to solve a structure of its complex with the ribosome recycling factor RRF,[86] and intermediates of the peptidyl transferase reaction.[87] Both we and the Noller laboratory used this crystal form to determine the structure of the ribosome in complex with release factors RF1 and RF2,[88–90] which shed light on translational termination. The same form has also been used in the study of elongation factor EF-P[91] and the antibiotics viomycin and capreomycin.[92] Despite its general usefulness, this crystal form was not suitable for the study of GTPase factors bound to the ribosome, such as EF-G or EF-Tu because of the particular packing in the crystal lattice.

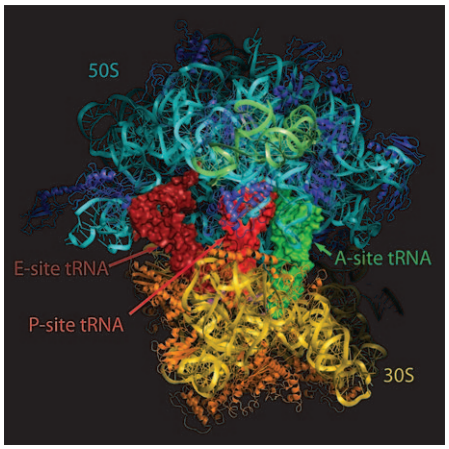

Figure 15. The entire 70S ribosome with mRNA and tRNAs, drawn from a structure at 2.8 Å.[85] The short section of mRNA in this structure is barely visible in magenta because it is buried in a cleft in the 30S subunit.

The Structure of the Ribosome with GTPase Factors

A characteristic of every crystal form of the ribosome previously studied was that regardless of the space group or species, protein L9 from a neighboring molecule in the lattice bound to the 30S subunit in such a way as to occlude the GTPase factor binding site. In the course of obtaining the high-resolution crystal form of the 70S ribosome described above, we learned that even with a stable complex like that of EF-G and fusidic acid with the ribosome, the L9 interaction was strong enough to displace the factor from the ribosome during crystallization. Thus over a period of many years, we were unable to obtain new crystal forms that were compatible with GTPase factor complexes of the ribosome as long as protein L9 was present. It was known that L9 was dispensable in *E. coli*,[93] so it was reasonable to assume this would also be true for *Thermus thermophilus*. Accordingly, we used genetic methods for *Thermus*[94] to delete the gene for the portion of L9 that protruded out of the ribosome. Subsequently we found that not even the remaining portion of L9 was not present in ribosomes, so it was as though we had deleted the entire gene for the protein.

Our initial hope was that this mutant ribosome would still crystallize in the same form as our high-resolution crystals[85] but be able to accommodate GTPase factors. Unfortunately, the absence of L9 prevented crystallization in this form. A broad screen to search for new conditions resulted in some hits for an EF-Tu complex with the ribosome, but these were not followed up because the people involved (Frank Murphy and John Weir) both left the laboratory.

Subsequently, after new crystallization trials from scratch, we obtained a new crystal form of the EF-G–fusidic acid

complex with the ribosome which turned out to be in very similar conditions to the original hits for the EF-Tu complex. These crystals diffracted to about 3.5 Å resolution and resulted in a structure of the post-translational complex with EF-G.[95] This structure revealed many details about the interaction of EF-G with the ribosome as well as the codon and anticodon. Because fusidic acid has very low affinity for isolated EF-G, the structure also directly showed how fusidic acid binds to EF-G and locks it in a conformation that prevents its release from the ribosome.

The Structure of the Ribosome with EF-Tu and Aminoacyl tRNA

The crystallization of the EF-G–fusidic acid complex with the ribosome provided the incentive to pursue the original hits with the EF-Tu–tRNA–ribosome complex. A considerable effort at optimization of these initial conditions resulted in crystals of the ternary complex bound to the ribosome stalled with kirromycin after GTP hydrolysis and the determination of its structure.[96] The overall structures of EF-G and EF-Tu bound to the ribosome are shown in Figure 16. The conformation of the factors is similar to what had been seen previously at lower resolution by cryoEM.

The EF-Tu–tRNA complex, in conjunction with earlier studies on the 30S subunit, provides a more complete view of the structural basis of decoding. The data suggest a sequence of events by which codon recognition eventually leads to GTP hydrolysis by EF-Tu and acceptance of the tRNA (Figure 17),[96] that can be correlated with kinetic data.[67,97] Recognition of the codon leads to an induced change in the three bases A1493, A1493, and G530 (Figure 17C), which recognize the minor groove of the codon–anticodon helix as seen previously.[72] This minor-groove recognition stabilizes the bent form of the tRNA in the complex by helping to hold the anticodon loop tightly in the decoding center. It also induces a closing movement of the shoulder towards EF-Tu, where it interacts with a highly conserved loop of EF-Tu and thus stabilizes the factor in an altered conformation (Figure 17D). At the same time, a base stacking interaction between the shoulder and C75 on tRNA stabilizes an altered conformation of the aminoacyl 3′ end of the tRNA. The displacement of the 3′ end of tRNA disrupts its stabilizing interactions with the crucial switch-I helix of EF-Tu, which then becomes disordered, exposing the GTP to hydrolysis. Upon GTP hydrolysis, EF-Tu changes conformation to its GDP form.[98–101] In the GDP form, it can make very few interactions with the ribosome and also has lower affinity for the tRNA, resulting in its dissociation from the ribosome and

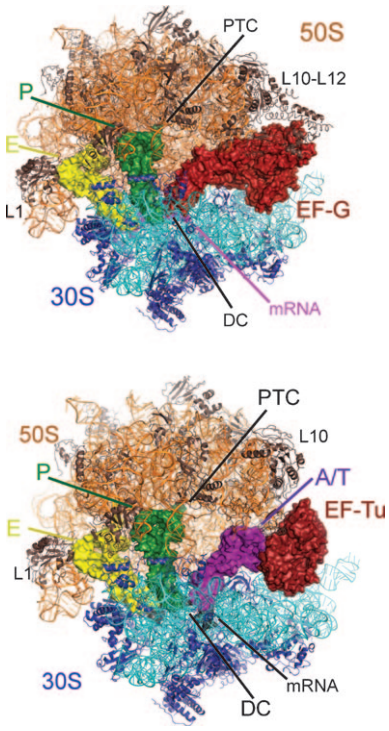

Figure 16. The structures of A) the GTPases EF-G and B) EF-Tu bound to the 70S ribosome.[95,96] The labels refer to the peptidyl transferase center (PTC) and the decoding center (DC), E-site tRNA (E), P-site tRNA (P), and the distorted tRNA in complex with EF-Tu in the A site (A/T). The L1 and L10–L12 stalks are also indicated.

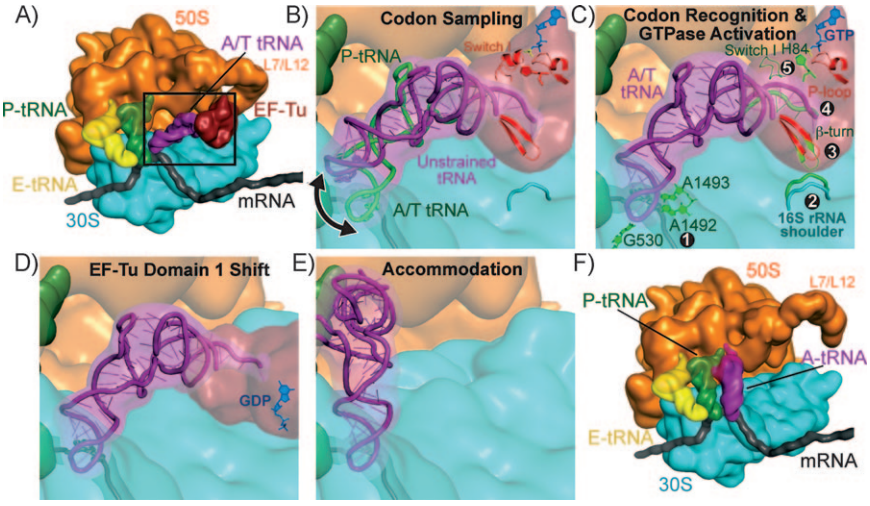

Figure 17. The decoding pathway leading to tRNA selection by the ribosome. A) The L7/L12 stalk recruits the complex of EF-Tu-GTP and aminoacyl-tRNA to a ribosome with deacylated tRNA in the E site and peptidyl-tRNA in the P site. The black frame represents the enlarged area in panels (B)–(E). B) The tRNA samples codon–anticodon pairing until a match (C) is sensed, by decoding center nucleotides 530 and 1492–1493 ①. Codon recognition triggers domain closure of the 30S subunit ②, bringing the shoulder domain into contact with EF-Tu, and shifting the β-loop at 230–237 of domain 2 ③. This changes the conformation of the acceptor end of tRNA ④, disrupting its contacts with switch I, which becomes disordered ⑤, opening the hydrophobic gate to allow His84 to catalyze GTP hydrolysis. D) GTP hydrolysis and Pi release cause domain rearrangement of EF-Tu, leading to its release from the ribosome and E,F) accommodation of aminoacyl-tRNA. Adapted from Ref. [96].

the movement of the distorted tRNA into the peptidyl transferase center (Figure 17E).

Future Prospects

The success in the determination of the high-resolution structures of ribosomal subunits and eventually the whole ribosome was the culmination of decades of effort. It was done in the context of a large body of important work using biochemistry and genetics that provided the intellectual framework for understanding the problem. It was made possible by many technical developments, not least the development of synchrotron radiation sources, better and faster computers, better software for crystallography, and better hardware and software for visualization and interpretation of the structures. The initial structures paved the way for the structures of a number of important functional states which have shed light on the mechanisms of various aspects of translation. They have also made possible increasingly sophisticated biochemical and genetic experiments, as well as molecular dynamics studies.

Nevertheless, many important structural questions remain. In bacterial translation, a detailed visualization of the steps in initiation remains to be determined, as well as the nature of intersubunit movements that are involved in translocation[102] and recycling. Understanding the interactions of the ribosome and the nascent chain with the signal recognition particle and the translocon will also be important. Beyond bacterial translation, ribosomes from eukaryotes or mitochondria remain more mysterious. In particular, understanding translational initiation in eukaryotes will benefit greatly from high-resolution structural information. In conjunction with other current work using a variety of techniques, these efforts will reveal fundamental insights into the regulation and mechanism of gene expression.

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