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From the Structure and Function of the Ribosome to New Antibiotics (Nobel Lecture)**

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antibiotics · Nobel lecture · protein synthesis · ribosomes

Autobiography

I was born in Milwaukee, Wisconsin, in 1940, and my family lived in an apartment above a paint store in the downtown area until 1949. Although my father had obtained a law degree from Marquette University in Milwaukee, he became the administrator in charge of personnel at the Milwaukee County Hospital. My mother grew up on a farm in Waukesha county outside of Milwaukee and graduated from Carol College, a small college in Waukesha. My mother devoted her time to all of the domestic chores required for raising a family which eventually grew to five children—two younger brothers and two younger sisters. My father's parents lived about 20 blocks away and my mother's parents and her brother's family lived on the family farm in Waukesha county.

I attended elementary school at the Elm Street School, an old brick building with an asphalt playground located a few blocks from our apartment. I did not like the school much and often got beaten up by a bunch of slightly older guys on my way home from school. My report card, which I brought home for my parents' signatures at the end of second grade, showed grades that were just above failure. My parents were upset and asked what I was going to do to change, and I said that I did not really care about the grades. My mother (I think) then applied the "board of education" to the "seat of knowledge"—my first and last spanking. This was definitely the low point of my academic career.

In the middle of my third grade school year we moved from 27th Street to a new house on 75th Street in the Milwaukee suburb of Wauwatosa and my life was transformed, academically and in all other ways. The teachers, the schools, the classmates were all marvelous in grade school, junior high, and high school. The Roosevelt grade school playground had tennis courts and a grass playing field, on which a large skating rink was made every winter with a warming hut and outside lights added. Almost every evening in the winter I would go skating for hours with friends playing team skating games.

Visits to my grandfather's farm during the 1940's and 50's played an important role in my life in that period. His farm was what is referred to as a "truck farm" where he grew vegetables, mostly radishes, carrots, and onions. In the World War II years, however, he had a cow for milk, cream and cheese and a picture from about 1941 shows me with my grandfather and his cow (Figure 1). My family made frequent visits to the farm, which was only about a 30 minutes drive from Wauwatosa. A picture of my parents and their five children standing in front of my grandfather's green Hudson car, taken in about 1952, shows a harvested wheat field and one of my grandmother's gardens (Figure 2).

Figure 1. I am in the arms of my grandfather standing next to his cow in a field on his farm, circa 1941.

During my early teenage years I spent most of my summer school vacation working in the fields on my grandfather's and uncle's farm. I worked with many other kids, bunching

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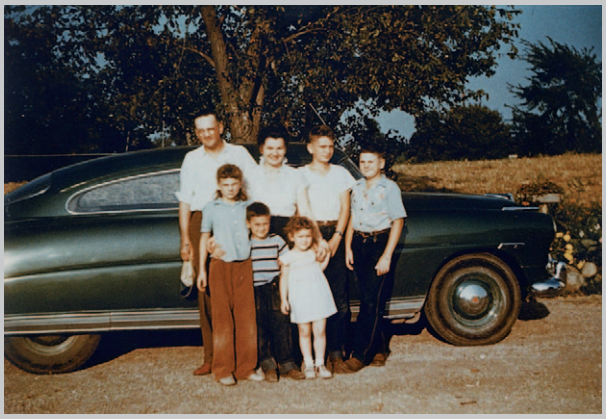

Figure 2. I am standing next to my father and mother who are on my right and my brother Dick, on my left. In the front are Mary, Bill and Sally (left to right), in about 1952. Behind us are my grandfather's 1951 Hudson and one of my grandmother's gardens.

radishes and weeding 20 acres of onions. Work would start just after sunrise at about 5:00 a.m. and continue to late afternoon every day, except Saturday. (The market was closed on Sunday.) I received 5 cents per dozen bunches of radishes and often tied as many as 100 dozen. I used the money to buy myself a new saxophone, a bicycle, a tennis racquet and save money for college.

The junior and senior high schools were about a 20 minutes walk away and introduced me to an additional set of classmates and importantly to music, art, and shop besides the academic courses. The shop courses in junior high included electricity and magnetism, where I made an electric motor from scratch, winding the wire coils and making all of the connections. In the woodworking shop I made a coffee table for the family. In the plastics shop I made letter openers and light stands. I do not remember what I did in metal working. I have found that the basic skills in working with tools and materials that I learned in the shop courses have proven invaluable for me in subsequent years, at home and in the laboratory, including constructing models of proteins. I think it is unfortunate that such courses have been eliminated in many schools today as being unnecessary or too expensive.

I developed a serious interest in music in junior high school where I joined the band and the choir. On the first day of band practice I brought my father's C melody saxophone to the band leader who told me it would not work in the band but lent me the school's E flat alto-saxophone. I became a very serious saxophone player and in high school played solos, duets, quartets as well as organizing a big band style dance band in addition to playing in the school band. I practiced one to two hours a day at home and won a number of "gold" medals at state contests when I was in high school. I seriously considered becoming a musician, but then concluded I could do music as a hobby if I went into science, but could not do science as a hobby if I went into music.

My grades in Longfellow Junior High School were mostly B's during my first two years—good but not great. Then, my younger brother Dick entered junior high during the middle of my second year, and he got straight A's. This was a wake up call for me and the competition was on. I believe I got mostly A's the next year. I did much better in high school, graduating 8th in a class of over 300. Competition can be motivating in the classroom as well as on the tennis court.

Fortunately, the students in all of my courses in high school were separated according to their academic ability in a particular field. Thus, the very best 25 to 30 students of the 300 total were in my math, science, and English classes. This, of course, meant that the teacher could instruct us at a much higher level than if the students in the classroom were a random selection from the whole class, and we learned from and were challenged by our fellow students. I particularly remember how extraordinarily good the girls were in my math classes—only two or three of the top ten were boys. I have never had any doubt that women are as good at or better than men in math, contrary to the impressions of a former president of Harvard. I think it is very unfortunate that many, if not most, high schools (like the one in my home town of Branford, CT) no longer separate students by ability; this does not motivate or properly educate the very best students.

Lawrence College

My choice of what college to attend was heavily influenced by my best high school friend, Alex Wilde, and most importantly, by his mother. My father wanted me to attend Marquette University or the University of Wisconsin, Milwaukee, neither of which appealed to me. I had gotten to know the Wilde family very well, particularly Alex's mother whose father was the then Senator Wiley from Wisconsin. She suggested that I apply to Lawrence College where Alex was intending to go, and since I could not afford the tuition, that I should apply for a scholarship, which I did. I received a full tuition scholarship for four years and upon visiting Lawrence College I knew where I wanted to go to college—an important choice.

My four years at Lawrence College changed my life, my view of the world, and my professional direction. Since Lawrence is a liberal arts school, I was required to take many humanities courses to supplement what turned out to be my major in chemistry. These courses began with what was called a Freshman Studies course which was a broad based reading, discussion and writing course on many classical books. We learned to ask as well as answer questions. Importantly, we were also required to take a philosophy course, a scholarly based (e.g., Niebuhr, etc.) religion course, and an anthropology course, as well as English, History, and language courses. I entered Lawrence with a heavy religious background and left it with an entirely different understanding of the origins of religious beliefs, their veracity, and their roles in cultures. Lawrence also has a music school so that I was able to continue my love of music by participating in the band, orchestra, and choir.

While I had many wonderful, inspiring teachers at Lawrence, the person who had by far the greatest influence in inspiring me to pursue a career in science, and in particular chemistry, was Professor Robert Rosenberg, or Bob as I can

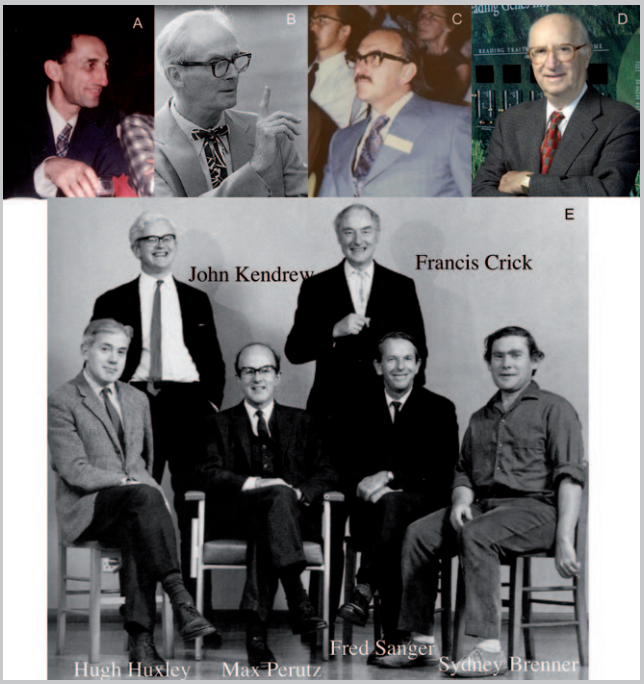

Figure 3. Some of the important mentors in my early career development: A) Bob Rosenberg, my chemistry professor at Lawrence College, B) William Lipscomb, my Ph.D. advisor at Harvard, C) Brian Hartley, my hexokinase pathfinder at the MRC Laboratory of Molecular Biology in Cambridge, England, D) Dan Koshland, Mr. "induced fit", E) the governing board of the LMB in the mid-1960s: John Kendrew and Francis Crick, standing, and Hugh Huxley, Max Perutz, Fred Sanger and Sydney Brenner (with cigarette), seated.

now call him (Figure 3a). I still recall the early lectures in his introductory chemistry course where he introduced to us the concepts of atomic orbitals and bonding and how studying chemistry at the physical chemical atomic level allowed us to understand the properties of chemicals, such as their color. It was a wonderful revelation to me about how the world around me could be understood.

I had several opportunities to work on research projects in laboratories outside Lawrence. The first opportunity, which was arranged by Bob Rosenberg, was to spend the summer between my junior and senior years doing research in the biochemistry laboratory of Lorazo Lorand at Northwestern University. My project was a kinetic study (determine k_{cat} and K_m) of the hydrolysis of a variety of *para*-nitrophenyl ester substrate analogues by chymotrypsin, trypsin, and thrombin. After making the measurements, I started the calculations, which seemed tedious. I then decided to write a computer program (my first) to process the data on the university IBM 650. The process went so quickly that I finished my summer project two weeks early and asked what I should do next. I was told to do some organic synthesis of a new substrate compound, which I began. While working in the hood with some organic solvents, in the presence of a lit Bunsen burner, the solvents, not surprisingly in retrospect, exploded (fortunately without any injury), and that ended the organic chemistry phase of my research career.

The end of summer before my senior year in 1961, I was invited to participate in a two week conference at Massachusetts Institute of Technology (MIT) for selected science students from small colleges. This unique meeting was organized and paid for by the American Biophysical Society in order to encourage students to consider the field of biophysics (of which I had never heard). Four students from about two dozen top small colleges were invited to participate, all expenses paid. It was my first trip on an airplane and my first trip outside of Wisconsin, except for Chicago. The venue consisted of lectures on a broad range of biophysical topics by faculty, mostly, but not exclusively, from Harvard and MIT. I remember one of the organizers, J. Oncley, sitting in the audience with the most wrinkled plaid sport coat I had ever seen. Most memorable, not only for its exciting content, was a lecture by Alex Rich. His lecture was truly inspiring and in an area of research I pursued years later. He was dressed in a fine dark suit (not today's lecture garb) with a white shirt and tie. Another important lecture for me was given by Paul Doty from Harvard on biophysical studies of nucleic acids, which was one of the reasons for my later wanting to attend Harvard. Students at the conference also had a great opportunity to interact with each other and go out to dinner together in various parts of Boston and Cambridge. I particularly remember dining with three Reed College students which included Don Engelman and Mark Ptashne, as well as a few others who subsequently became fellow graduate students at Harvard. This two-week meeting was possibly inspired in part by the Kennedy call to respond to Sputnik. It was a truly important event for influencing the career choices of many of us, but unfortunately was not repeated.

In the fall of my senior year I participated in a research program for selected students sponsored by the Midwestern small colleges at Argonne National Laboratory. I lived on the lab grounds and worked on a chemistry project that I neither liked nor remember. All I remember of the dull semester was my first opportunity to see the Moscow Bolshoi Ballet and marveling at the ability of the male dancers to leap across the stage. During the summer between Lawrence and starting studies at Harvard I worked for Dupont on another forgettable project, measuring the dynamic stretching modulus of various synthetic cloth materials being considered for use in making bras. I, unfortunately, was not invited to join the group that evaluated the final product being modeled.

Harvard University

I went to Harvard as a graduate student to work on biophysical studies of nucleic acids, but fortunately, chose a different pathway. In the spring of my first year in 1963, I attended three Dunham lectures given by Max Perutz (Figure 3E) in which he presented the first atomic resolution protein crystal structure, that of myoglobin. He showed stereo slides, and I was stunned to see the atomic structure of myoglobin pop out in three dimensions over Max's head; this was clearly the way to understand how macromolecules carry out their biological functions. Shortly thereafter, while play-

ing tennis with a Lipscomb graduate student (Peter Boer), I mentioned how unfortunate it was that no one was doing protein crystallography at Harvard. He said on the contrary, the "Colonel", as Bill Lipscomb (Figure 3B) was referred to by his students and postdocs since he was from Kentucky, had a group who were working on the crystal structure of bovine carboxypeptidase A (CPA). Shortly thereafter I made my way to the Colonel's office, a little nervous because I had already been turned down (fortunately) by another faculty member, in order to make an appointment to see him. He was standing in the office of his secretary, who was not there, and when I asked if I could make an appointment to see him, he invited me right in. After he described the CPA project, I excitedly asked if I could join the project, and he said yes. So, I had just received the wonderful opportunity to join the Colonel's army, and the rest is history.

The CPA team consisted of five postdocs at that time, of whom Martha Ludwig was the most important for my training. I worked with her on structural studies of substrate and inhibitor complexes of CPA as well as with the whole group on the determination of the crystal structure of the apo protein. I remember being excited at one time when we succeeded in collecting 5000 reflections in one week using the Hilger–Watts linear diffractometer. Now, of course, we can collect 5000000 reflections from ribosome crystals of the ribosome in an hour (about a factor of 10^5 faster for an assembly that is about 80 times larger). All computer programs were written in Fortran for an IBM 7094 computer that had 32 K of memory, had no discs and used computer cards. The advantage of the latter was that we could save the used cards and sell them as used paper in order to support lab parties every few months. (I always thought we should have written a program called GENCARD to increase the party frequency.)

Because of the superb team that the Colonel assembled and his encouraging management style, the project moved on well. In 1966 we published a 6 Å resolution map, including a model of the polypeptide backbone that I had over-optimistically built, but showing many of its structural features. Martha and I, with a postdoc Flo Quiocho, published a low-resolution map of an inhibitor complex that showed the first example of a substrate-induced conformational change. In 1967 we obtained what I realize in retrospect was a superb 2.0 Å resolution electron density map of the apo-CPA that allowed us to correctly position every residue of the polypeptide backbone, even in the absence of an amino acid sequence. CPA tied in 1967 with three other proteins, RNase A, RNase S and chymotrypsin, for being the third high-resolution protein structure determined after myoglobin and lysozyme.

Unlike principal investigators today, the Colonel was almost never absent from the lab to attend meetings and present seminars. He did, however, take a sabbatical in England, and while he was gone, I came up with the idea of using direct methods to phase the $F_{\text{derivative}}-F_{\text{native}}$ difference coefficients in order to calculate a projection difference Fourier map, which clearly showed the heavy-atom positions. When I showed him the paper I had written on the work upon his return, he allowed me to publish the paper in *Acta Crystallographica* without his being a co-author, also not a common practice among PIs today.

The Colonel provided me with what turned out to be a great opportunity that had an important impact on my future faculty job opportunities when he arranged for me to give a talk at the Protein Gordon Conference, which was chaired by Fred Richards in the summer of 1966. I talked about the CPA structure and the conformational change produced by substrate or inhibitor binding, the concept of induced fit. Dan Koshland was a participant and greatly appreciated this new experimental evidence for his hypothesis of substrate-induced conformational changes. I assume that my talk and opportunity to meet him partly motivated his advocating that the Berkeley Biochemistry Department interview me and offer me a faculty position, which they did, and I accepted in the late spring of 1967. Due to the reluctance of the Department to consider hiring a woman for a faculty position (Joan) in 1970, I resigned my Berkeley position after two months on the faculty and accepted a position at Yale offered by Fred Richards.

The Laboratory of Molecular Biology, Cambridge

After Harvard and before going to Berkeley I spent 3 years at the Medical Research Council (MRC) laboratory of Molecular Biology in Cambridge, England, from 1967 to 1970 in the group of David Blow. He was recommended to me by Hilary Muirhead, who was a postdoc with the Colonel and a former student with Max Perutz. In David's lab I worked with Richard Henderson on determining the structure of chymotrypsin complexes with substrates.

The Cambridge Laboratory of Molecular Biology (LMB) was a completely unique and outstanding laboratory. It inspired and trained a very large group of post-docs from the U.S. in molecular and structural biology who then returned and transformed these fields in the U.S. Perhaps the most remarkable and unique feature of the laboratory was the canteen located on the top floor which provided coffee in the morning, lunch after mid-day and tea in the afternoon. The attraction was definitely not the "bangers" or, the "toad in the hole" or other culinary opportunities, but sitting down with a random collection of lab directors, post-docs, and graduate students and talking about science. The canteen was set up by Max and run by his wife, Gisela. When I first arrived, it was so small that whenever I got through the food line, there were only a few empty seats. Consequently, I would have to sit at a table that might include Max, Francis Crick, and Sydney Brenner (Figure 3E) at it as well as post-docs and students. Within about two months I had met nearly everyone in the whole laboratory. The conversations were always about science and about experiments, never about the movie someone saw the previous night. Everyone contributed suggestions and/or criticisms. Initially I wondered how anyone got any experiments done since they were spending so much time in the canteen, and then I realized that the many discussions reduced the number of unwise or unnecessary experiments that were done and enhanced the good ones.

There were no weekly group meetings, but there was an annual one-week meeting for essentially everyone in the LMB which was generally known as "Crick week". Francis would sit in the front row and frequently ask many questions. On one occasion I remember Fred Sanger presenting a talk on his recent research and in the middle Francis jumped up and said "*Fred, if you did (this) and (this) and (this), then you would be able to find out (that) and (that)*". Fred, without taking his hand off the chalkboard turned toward Francis and said, "*That's it, Francis, that's it; you've got it*", and then carried on. Since Crick week included a broad range of molecular and structural biology topics, it was very useful that directors in the front row would ask questions that many of us were reluctant to ask. During one lecture that involved a comparison of a process that occurs both in eucaryotes and procaryotes, Max asked, "*What is a eucaryote and what is a procaryote?*", terms that were just beginning to be used. I was glad that Max asked the questions, since I had no idea what the terms meant. Sydney Brenner would have coffee available in his lab dishwashing kitchen on Saturday morning about 10:30 or so and would always be there with the post-docs and students from the molecular biology floor directed by himself and Francis, plus others of us who wanted to drop by. Sydney would invariably hold forth on some interesting topic with lots of funny stories and "one-liners".

I learned about all of the major research problems being pursued at the LMB from Crick week, the canteen, Saturday coffee, and the random conversations in the hall, as well as the quick evening trip to the pub for "last call", which the American post-docs did. (The Brits were mostly not there at night.) It was at this time that I developed my interests in trying to understand the structural bases of the mechanisms by which the many proteins and nucleic acids that are involved in "Crick's Central Dogma" carry out their functions: how DNA is copied into DNA, DNA transcribed into RNA, and finally the RNA translated to protein.

Access to computing facilities was extremely limited at Cambridge. The LMB used the computer owned by the Cambridge University astronomy department, and we were allowed to make only two submissions a day for the five working days, and the morning run could not take more than two minutes. I would check and recheck the computer cards I was submitting to eliminate as many mistakes as possible, because otherwise one of ten opportunities would be lost to me for the week. In retrospect, it is amazing that we were able to accomplish anything. Certainly, the timescale was longer.

At some point in my second year Brian Hartley (Figure 3C), who was collaborating with David Blow on chymotrypsin studies, came up to me and asked what research project I planned to pursue when I left the LMB and went to Berkeley. I said I wanted to solve the structure of an aminoacyl-tRNA synthetase, ultimately complexed with substrates including tRNA. This is the step where a specific amino acid is attached to the tRNA containing the correct anticodon. Brian patted me on the back and said "*There, there, my boy. That is an interesting problem, but you must work on something you can actually do successfully. I suggest that you study the structure of hexokinase*". I thanked him for the advice and ran down to the library to find out what hexokinase was. I subsequently learned by reading papers from Dan Koshland (Figure 3D) that the hexokinase reaction was his primary example of why some enzymes must undergo a substrate-induced fit conformational change. He reasoned that if the enzyme was rigid with all of its catalytic groups properly oriented to catalyze the nucleophilic attack of the 6-hydroxy group of glucose on the alpha phosphate of ATP, then why would water not hydrolyze ATP in the absence of glucose? The 6-hydroxy group is after all a water molecule with some carbons attached. He hypothesized that the binding of glucose must cause a conformational change in the enzyme that is necessary for catalysis. I consequently started growing crystals of hexokinase at Cambridge and spent the next 10 years studying this enzyme. Mentorship is always essential, and not only from your direct supervisor.

I submitted my first grant application to the National Institutes of Health (NIH) in 1969, I believe, in which I proposed to determine the structures of yeast hexokinase, of which I had managed to produce crystallographically suitable crystals. I had to go to Berkeley for a site visit by an NIH panel and was asked by a panel member how I was going to collect the X-ray data. I said by using a diffractometer, whereupon I was told that data collection with a diffractometer would not work because the unit cell dimensions of my hexokinase crystals were too big (one dimension was 200 Å). This, of course, was a ridiculous comment, since one just moves the detector further away from the crystal, but the reviewer was firm and ultimately my first application was turned down. (A few decades later this reviewer asked me in an elevator while being escorted to my next faculty visit how we had solved the structure of hexokinase, and I said "*by using a diffractometer to collect data*".)

Sometime at the end of 1969 or early 1970, Fred Richards (Figure 4) was visiting the LMB, and I had an opportunity to talk to him. Since I had become worried about my funding at Berkeley, I asked him whether a faculty position for me was possible at Yale, and he said he would look into it and get back to me. Years later I learned from Sydney Brenner, while dining with him at King's College, that Fred had run up to his office after talking to me and asked him if he could encourage another American postdoc in Sydney's lab to either accept or reject the offer that he had received from Yale. Sydney immediately called the postdoc into his office, closed the door, put a piece of paper on the table and told the postdoc that he would not be allowed to leave the room until he had written and signed a letter to Fred either accepting or rejecting Yale's offer. Sydney then took the letter rejecting Yale's offer to Fred, who left Cambridge with two faculty slots in his pocket. Fred had wanted to have two positions available, since there were faculty in the department who wanted to hire Joan, and perhaps Fred thought Yale might have an advantage over Berkeley if they offered both of us jobs. Indeed, that turned out to be true.

Yale

I arrived at Yale in the late fall of 1970 and began our structural studies of yeast hexokinase captured with and

Figure 4. The faculty who were members of the Yale Center for Structural Biology in 1995 when our work on the ribosome began. On top are HHMI investigators Jennifer Doudna, Paul Sigler and Axel Brünger (left to right). On the bottom is the WERMS group in 2001: Hal Wyckoff, Fred Richards, myself and Peter Moore (left to right) in the back row and Don Engelman in front.

without the substrate glucose bound, a project that occupied the efforts of most of my lab during the 1970's. I was extremely fortunate to have Robert Fletterick join my lab to work on hexokinase as my first postdoc during my first year at Yale. Bob had come to Yale to do a postdoc with Hal Wyckoff and had a fellowship. He decided he wanted to switch labs, and Hal was very accommodating. Our structures of hexokinase with and without glucose bound showed the largest conformational change in a single subunit that had been observed at that time and clearly established that Koshland's induced-fit hypothesis was correct for explaining the specificity of hexokinase. The pictures of our hexokinase structures with and without glucose bound have been published in far more textbooks than any other work from my lab. Brian Hartley had certainly made a good suggestion for what research direction I should pursue, and I can only wonder what I would have accomplished in the 1970's had I not had the hallway hexokinase discussion with Hartley.

A very important factor in making the quality of structural biology so excellent at Yale beginning in the 1970's was the shared computation and X-ray facility, the "core" laboratory, and the many interactions it facilitated. When I arrived in 1970, Fred Richards and Hal Wyckoff, who solved the structure of RNase S in 1967, had a shared X-ray and computation lab that I joined and added some equipment. In 1975 I suggested to Fred that we should consider applying for an NIH program project grant, which I had just learned about, to support our structural biology efforts, and Fred took the lead in organizing five of us, the WERMS group (Wyckoff, Engelman, Richards, Moore, and Steitz), to apply which we did successfully in 1976. The "WERMS" grant, as we referred to it, is now in year 34, but I am the only WERM left on it. In the late 1980's, funding from HHMI provided additional support for the core X-ray and computational lab, including two technical staff positions and additional equipment. They also provided an investigator position for me as well as additional faculty/investigator positions for the Molecular Biophysics and Biochemistry (MB&B) department, and in the mid-1990's the WERMS group also included Paul Sigler, Axel Brünger, and Jennifer Doudna (Figure 4).

This group of seven laboratories constituted the Yale Center for Structural Biology (CSB) and Fred Richards was appointed by the Yale president to be the first director of the CSB. The shared core computation and diffraction laboratory was always abuzz with the activity of students, postdocs and technical staff who interacted and helped each other solve problems. In the mid-1990's there were six technical staff in the core lab to help users with problems they encountered, and about 100 postdocs, students and technical staff in the CSB laboratories. All of the seven faculty members of the CSB in 1995 (when our work on the ribosome began) are or were in the U.S. National Academy of Sciences. In the mid-90's, these seven labs and their extensive and collegial interactions provided perhaps the best environment in the world for doing structural biology in general and determining the structure of the ribosome in particular.

Son Jon

Our son Jon was born in 1980 and met his first Nobel prize winner, Fred Sanger, at the age of 4 weeks during an MRC LMB celebration on the day of Fred's being awarded his second Nobel Prize in chemistry. We happened to be in Cambridge on that day, after attending meetings in Switzerland, Germany, and London. Jon got to go to many meetings around the world for the next fifteen years until baseball took over his world.

I started playing tennis and baseball with Jon when he was in grade school and installed a basketball hoop on the garage for him. Every weekend in the summer we would go to a baseball field in our home village of Stony Creek to practice his throwing, catching, and batting skills. That lasted until he started hitting the ball out of the park into the salt marsh grass. In high school he was quarterback on the football team, guard on the basketball team, and pitcher on the baseball team for four years. At the end of his senior year he was drafted in the 44th round of the baseball draft, but wisely chose to go to Yale. At Yale he majored in molecular biophysics and biochemistry, and baseball, as did two of his classmates and teammates, Craig Breslow and Matt McCarthy. At the end of his junior year, Jon was drafted by the Milwaukee Brewers (ironically) in the third round and received a signing bonus that was slightly larger than my share of the 2009 Nobel prize. After a shoulder injury caused him to leave baseball, he went to Yale Law School and is now working in consulting with McKinsey Corporation. Breslow, who worked in Joan's lab as an undergraduate and intended

to go to medical school had also been drafted by the Brewers and is now pitching for the Oakland Athletics team.

Jon learned to ski by coming on what we now refer to as "Riboski" trips. Starting in the late 80's, a group of RNA-centric friends and their kids started going on annual ski trips together: Tom and Carol Cech (+2); Jim and Elsbet Dahlberg (+2); John Abelson (+1) and Olke and Lori Uhlenbeck. Since we sent the kids to take ski lessons while the adults skied together, Jon quickly improved, and by the age of 12 he could ski circles around me. Perhaps my most memorable ski trips were my two 4 day trips with Jon to Snowbird in Utah over Thanksgiving break during his junior and senior years in high school (Figure 5). He would ski the double black diamond runs while I would ski the double blue or black diamond trails and we would meet at the bottom of the ski lift.

Figure 5. Son Jon and myself on our ski trip on an Alta Ski trail near Snowbird in Utah over Thanksgiving break in November, 1997.

The Structural Basis of Crick's Central Dogma of Molecular Biology

Our decades' long quest to obtain a structural understanding of the mechanisms by which the macromolecules that carry out the process of DNA makes DNA makes RNA makes protein—Crick's central dogma—began with our establishing the structure of the catabolite gene activator protein (CAP) with only cyclic adenosin-3′,5′-monophosphate (cAMP) bound in 1981. This was the first structure of a DNA binding protein, a transcription activator. Our subsequent structure of CAP bound to DNA in 1991 showed a remarkable bending of the DNA backbone, and our recent structure of the unliganded CAP exhibited a very large conformational rearrangement of the DNA binding domains which explains how the binding of cAMP activates the ability of CAP to bind to DNA.

In the 1980's we also determined the first structure of a DNA polymerase, the Klenow fragment of DNA polymerase I (whose discovery by Arthur Kornberg led to his receiving the Nobel Prize) and its complex with a DNA substrate in the 3′,5′ exonuclease active site. The structure of the substrate complex led to our discovery of the two-metal-ion mechanism of a phosphoryl transfer reaction and our later proposal that this mechanism is employed by many ribozymes, which has recently shown to be the case for several of them. We also published our first structure of a fragment of the site specific recombination enzyme, gamma delta resolvase, that lacked its sequence specific DNA binding domain. Perhaps the most exciting (to me) leap forward in the late 80's was our obtaining the structure of glutaminyl-tRNA synthetase complexed with $tRNA^{Gln}$ and ATP. This was the problem I had wanted to work on 20 years earlier when Brian Hartley wisely advised me that it was too early. Obviously, he was correct. This first structure of a synthetase–tRNA complex showed how the synthetase recognizes the correct tRNA containing the glutamine anticodon and discriminates against all of the other tRNAs. This is the first critical step in the translation of the genetic code into proteins.

We had many exciting advances in our central dogma quest in the first half of the 1990's. We obtained the first structure of HIV reverse transcriptase complexed with a non-nucleotide inhibitor, which was then one of the few drugs used to treat patients with AIDS. We also determined the first of the many structures of T7 RNA polymerase that we have obtained over the last 15 years captured in many of its functional states. This was the beginning of our exploring how DNA is transcribed into RNA starting with an initiation state with T7 RNA polymerase bound to its promoter and going on to the elongation and termination states. Significant progress was also made in our studies of DNA recombination. We obtained the first structure of an enzyme involved in homologous recombination, recA, and the structure of the site specific recombinase, gamma delta resolvase, bound to its specific DNA target. While this latter structure illuminated how resolvase recognizes its DNA target, it did not reveal how the protein brings the two DNA duplexes together to form a synaptic complex or how strand exchange is accomplished; that would take us another 10 years. We also obtained the structure of the first binary complex of a DNA polymerase (Klenow fragment) with its duplex DNA substrate bound to the polymerase active site, but without the incoming dNTP.

By 1995, then, we had made significant progress on obtaining structural insights into the mechanisms of all of the steps of the central dogma except the last one: protein synthesis by the ribosome. It was at this time in the fall of 1995 that Nenad Ban joined by lab and said he wanted to work on the structure of the ribosome—the right person at the right time. As discussed in more detail in the Nobel lecture, we collaborated with Peter Moore and Ban was joined later by Poul Nissen and Jeff Hansen. Between 1995 and 2000 our goal of obtaining the structure of the 50S ribosomal subunit and a complex with a transition state intermediate was attacked successfully by the "swat team" of these three postdocs (Figure 6). Jeff Hansen also determined the structures of many complexes between the *Haloarcula marismortui* 50S subunit and antibiotics bound to the peptidyl transferase center, which formed the basis for our founding of Rib-X Pharmaceuticals, Inc. Subsequently, substrate complex structures were pursued by a graduate student, Martin Schmeing,

Figure 6. Some of the key team players in the ribosome project shown enjoying a reception given by the Nobel Foundation at the Nordic Museum, Stockholm, on December 9, 2009. From left to right are Poul Nissen, myself, Peggy Eatherton, Peter Moore, Nenad Ban, Martin Schmeing, and Jeff Hansen. Peter is my long time faculty colleague, friend and collaborator on the ribosome project. Poul and Nenad are former postdocs in the lab who were the ones primarily responsible for determining the structure of the 50S subunit. Jeff is a former postdoc whose major contributions included the structure of antibiotic and substrate intermediate analogue complexes with the 50S subunit. Martin is a former graduate student whose many structures of substrate analogue complexes that were captured in the various steps of catalysis allowed him to make a movie of peptide bond formation on the 50S subunit. Peggy has been my administrative assistant for 25 years and has been an enabling facilitator, memory chip and coordinator of lab personnel.

in the early 2000's (Figure 6). During the 1990's our small ribosome group had daily conversations and regular meetings around a lunch table to discuss progress and ideas for moving forward. The calculation of the 2.4 Å resolution electron density map in early 2000 and our months of building a model of the ribosome were the most exciting research times I had ever experienced. We had no idea what the ribosome structure, particularly the RNA, would look like and peering into its emerging interior was simply amazing.

Looking back over the development and progress of my career in science I am reminded how vitally important good mentorship is in the early stages of one's career development and constant face-to-face conversations, debate and discussions with colleagues at all stages of research. Outstanding discoveries, insights and developments do not happen in a vacuum. Our research accomplishments on the structures of the large ribosomal subunit and its many complexes were greatly enhanced and accelerated by the structural biology environment at Yale in the 1990's as well as the long term support of risky projects by the Howard Hughes Medical Research Institute. As I watch increasing numbers of my faculty colleagues, students and postdocs communicate with each other almost exclusively by email rather than discussing ideas over the lunch table (as I experienced in Cambridge and the first decades at Yale), I wonder whether they will be as creative and have as much fun doing science as they could with more face-to-face contact.

Nobel Lecture

My passion for pursuing structural studies of biological macromolecules in order to understand how they carry out their functions was initiated by a Dunham lecture that Max Perutz presented at Harvard Medical School in the spring of 1963, a year after he shared the Nobel Prize in Chemistry with John Kendrew for determining the first protein structures. He showed a very large audience the first stereo slide of an atomic structure of a protein, myoglobin, that any of us had ever seen. When the myoglobin structure popped into three dimensions over his head, a loud "oh" came from the audience. I knew then how I wanted to understand the chemistry of biology.

I began my thesis research at Harvard by working with a team in the laboratory of William N. Lipscomb, a Nobel chemistry laureate in 1976, on the structure of carboxypeptidase A. I did postdoctoral studies with David Blow at the MRC lab of Molecular Biology in Cambridge studying chymotrypsin. My interactions with Jim Watson and with Wally Gilbert while I was at Harvard and the numerous contacts that I had with Francis Crick and Sydney Brenner while I was at Cambridge stimulated my three decades' long interest in obtaining the structural basis of Crick's Central Dogma: "*DNA makes DNA makes RNA makes Protein*". This trail ultimately led to our determining the atomic structure of the large ribosomal subunit, which catalyzes peptide bond formation, as well as the structures of its complexes with substrate analogs and antibiotics.

In the early 1960's, when I was a graduate student, Watson published a figure that summarized what was known about the ribosome structure.[1] It showed the A site for the positioning of the aminoacyl-tRNA, though nothing was known about the tRNA structure. The P site located next to the A site had the peptidyl-tRNA, but the pathway taken by the polypeptide product was unknown. Also, the existence of the E site, the exit site, was unknown. In 1976, Jim Lake used electron microscopic studies of negative large and small ribosome subunits as well as the 70S ribosome to obtain the first views of the shapes of the ribosome and its subunits.[2] By 1995 Joachim Frank was able to use the single particle cryo-EM methods that he and co-workers had developed to obtain a 25 Å resolution reconstruction of the 70S ribosome with three bound tRNA molecules.[3]

By 1995, my lab had obtained structural insights into the mechanisms of most of the steps of the Central Dogma, except the last one: protein synthesis by the ribosome. The mid-90's seemed to be the right time to take on this largest of structural biology challenges. Computational power and X-ray crystallographic methodologies including synchrotron X-ray sources and CCD detectors had reached a sufficiently high level to allow X-ray data collection from crystals of such a large assembly. Importantly, Ada Yonath and Wittmann had shown in 1985 that the 50S ribosomal subunit could be crystallized,[4] and in 1991 crystals of the *Haloarcula marismortui* (Hma) 50S subunit were obtained that diffracted to 3.0 Å resolution.[5] The growth of these well-diffracting crystals meant that obtaining the atomic structure of the ribosome was in principle possible. However, while crystals

are a necessary condition for determining a crystal structure, they are not sufficient: a large challenge remained—the phasing problem. The 7 Å resolution electron density a map of the Hma 50S subunit that was published in 1995[6] suggested to me (and some others) that the challenge had not yet been correctly met, since the map did not look like RNA. Another approach was needed.

In the fall of 1995 Nenad Ban joined my lab and was interested in pursuing the structure of the ribosome or its component large subunit—the right person at just the right time. I suggested that he tackle the Hma large subunit structure, which he did. I also decided that we should collaborate with a close friend and colleague, as well as one of the pillars of the ribosome research community, Peter Moore. Peter is an avid fisherman who likes to catch big fish, and the ribosome was indeed a big fish. Nenad embarked on determining the structure of the Hma 50S ribosomal subunit with the assistance of Peter's technician, Betty Freeborn, for preparing the subunit. A student in Peters's lab concurrently pursued the objective of crystallization of the 30S subunit or domains of it. By early 1997, Nenad had successfully initiated very low resolution crystallographic studies of the large subunit including the correct location of the heavy atoms in several heavy-atom derivatives, when he was then joined in his efforts by Poul Nissen. Through the next three years these two spearheaded the structure determination of the Hma 50S subunit.

While the crystals obtained using the published procedures[5] diffracted to 3 Å resolution, they were extremely thin and often multiple. Indeed, Yonath and Franceschi (1998)[7] and Harms et al. (1999)[8] described these crystal defects, which included severe non-isomorphism, high radiation sensitivity, nonuniform mosaic spread, uneven reflection shape, and high fragility, as well as unfavorable crystal habit. Nissen introduced a back extraction procedure that resulted in isometric and uniform crystals that occasionally diffracted to 2.4 Å resolution.[9,10] Later, Martin Schmeing found an approach that extended the resolution to 2.2 to 2.4 Å more reproducibly.[11,12] At this resolution the structures, when obtained, can inform on the chemistry of the processes involved in protein synthesis.

What then was the major challenge that needed to be overcome? Why was the determination of the atomic structure of the ribosome perceived to be a very high mountain to climb? The major challenge in determining any crystal structure (once crystals have been obtained) is what is called the "phase problem". Each diffraction spot has an intensity, which can be directly measured, and a phase, which is not directly measurable. Max Perutz was awarded the Nobel Prize in 1962, in part because he developed the method of heavy atom isomorphous replacement to solve the phasing problem for macromolecules. Heavy atoms are bound specifically to the crystal, and their positions in the crystal need to be determined, information that can then be used to obtain the phase angles, which when combined with the diffraction amplitudes allow the calculation of an electron density map.

The phasing challenge presented by the ribosome arises from its large size. Consequently, a single heavy atom provides too weak a diffraction signal to measure and 100 heavy atoms are difficult, if not impossible, to locate. I compare the problem with the challenge of trying to measure the weight of a ship captain by subtracting the weight of the boat from the weight of the boat plus the ship captain. While this can be done with some accuracy for a small sailboat, subtracting the weight of the Queen Mary from that of the Queen Mary plus the captain would give a very small signal, and the ribosome is the Queen Mary of macromolecular assemblies. It is about 100 times heavier than lysozyme.

In order to obtain a super heavy ship captain, Ban used several heavy atom cluster compounds, most importantly one containing 18 tungsten atoms (W_{18}) which together with the other atoms in the compound has about 2000 electrons. At very low resolution, 20 Å or lower, it scatters almost as one heavy atom. Since the X-ray scatter is proportional to the square of the number of electrons, the scattering signal from the W_{18} cluster compound is over 600 times larger than that from a single 78-electron tungsten atom. Indeed, its scatter at low resolution is very much larger than that from more than 100 bound osmium hexamine complexes (Figure 7).

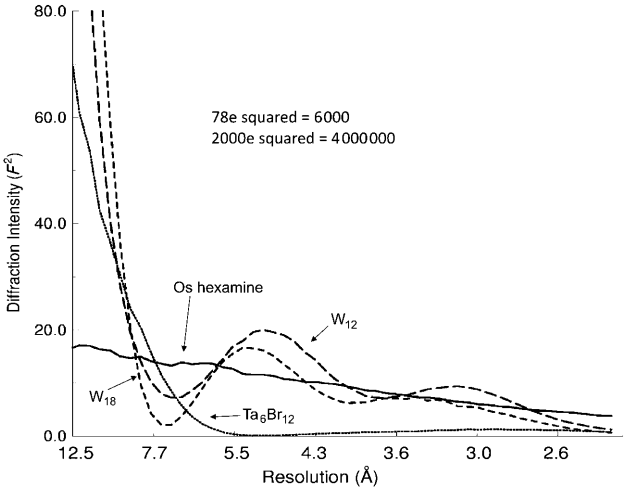

Figure 7. The calculated radial distribution of the scattering intensities produced by four of the heavy-atom compounds used for phasing as a function of resolution. At very low resolution the scattering from the cluster compounds, including the W_{18} cluster which contains 2000 electrons, is extremely large compared with the scatter from more than 100 bound osmium hexamines.

Nenad Ban located the position of a W_{18} cluster compound that was bound to a single site using a 20 Å resolution difference Patterson map.[13] He then confirmed its location by calculating a difference electron density map, phased using molecular replacement phases derived from a 20 Å resolution cryo-EM map of the Hma 50S subunit provided by Joachim Frank (Figure 8, top left). Ban then solved several additional heavy-atom cluster compound derivatives using phases derived from the W_{18} derivative, and by the end of 2007 he had a very nice 9 Å resolution map of the 50S subunit (Figure 8b, top right), obtained using only X-ray data, that showed the expected RNA duplex helices and had the same overall shape as seen in the cryo-EM map.[13]

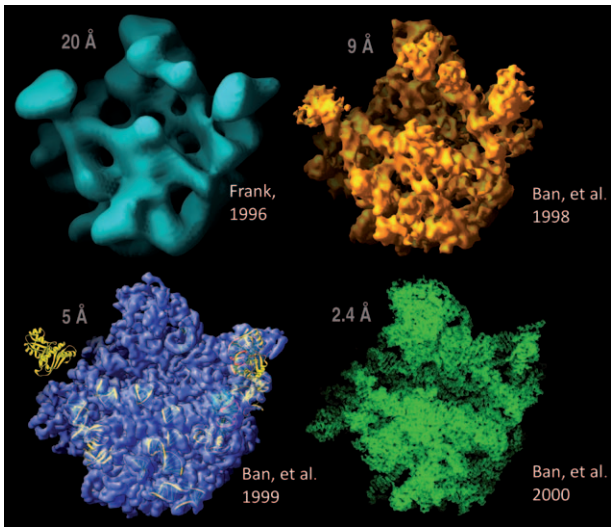

Figure 8. The progressive increase in the resolution of the electron density maps of the 50S ribosomal subunit obtained, beginning with the 20 Å resolution cryo EM map from Joachim Frank (1996) and progressing to our 9 Å resolution map,[13] which showed the first of the RNA helices, to the 5 Å resolution map,[9] into which known protein structures could be fitted, and ending with a 2.4 Å resolution map,[10] which allowed the building of a complete atomic model.

Our strategy during the first four years of our crystallographic studies of the 50S subunit was to work at lower resolutions than 4.5 Å, which could be done using a bending magnet beam line X12C at Brookhaven National Laboratory on Long Island, New York. It was not possible to use the laboratory rotating anode X-ray source because it was too weak, but the X12C source worked fine at low resolutions and was generally very accessible for our use. When finally all of our heavy-atom derivatives were made and the heavy atoms correctly located, our first trip to the high intensity insertion device beam line X25 at Brookhaven was made at the end of 1999 and within four days data were collected that allowed calculation of a 3.0 Å resolution map and the initiation of the building of the atomic model.

The resolution of our maps gradually increased from the initial 9 Å resolution (Figure 8). In 1999, we published a 5 Å resolution map of the 50S Hma subunit in which known r-protein structures could be positioned (Figure 8c, lower left).[9] In 2000, we published the atomic structure of the 50S ribosomal subunit derived from a 2.4 Å resolution map calculated using data collected at Argonne National Laboratory[10] (Figure 8, lower right) and that of its complex with a substrate analogue of the transition state of the peptidyl transferase reaction.[14] At the same time in 1999 that we published our 5 Å resolution map, the Ramakrishnan group published a 5.5 Å resolution map of the 30S subunit,[15] and the Noller group published a 7 Å resolution map of the 70S ribosome,[16] using phasing approaches that were similar to the cluster approach we published in 1998. Shortly after the appearance of our papers on the 2.4 Å resolution structures of the 50S subunit, two models of the 30S subunit were published.[17,18] A year later Noller and colleagues obtained a model of the 70S ribosome with three bound tRNA molecules using a 5.5 Å resolution map into which were fitted the atomic models of the 30S subunit of Ramakrishnan et al. and the Hma 50S subunit modified to reflect the eubacterial differences.[19]

The 3000 nucleotides of RNA observed in the Hma 50S subunit exhibited a compact structure with the globular domains of the r-proteins imbedded in its surface, except in the deep cleft where the substrate analogue binds. Splitting the subunit down the middle like an apple and opening it out reveals a 100 Å long polypeptide exit tunnel emanating from the peptidyl transferase center (PTC). It is wide enough to accommodate an α-helix,[14,20] but not large enough to accommodate any formation of protein tertiary structure as had been proposed.[21] Not only is the packing of the 23S rRNA relatively tight, but extended peptides from many r-proteins are seen to fill the crevices that lie between the RNA helices (Figure 9). Indeed, when the structures of many of the

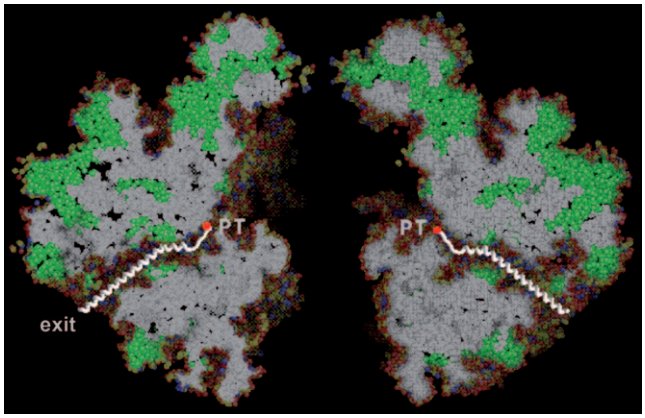

Figure 9. A space filling model of the Hma 50S ribosomal subunit cut in half through its polypeptide exit tunnel at the PTC (PT) and opened up like a book. The tightly packed RNA in the interior is shown in white and the penetrating protein loops in green. A hypothetical model of the exiting polypeptide in the tunnel is shown in white.[14]

r-proteins are examined in isolation, they are seen to consist of globular domains and idiosyncratically folded extended loops and strands that contain many Lys and Arg residues. Two particularly striking examples of extended chains that penetrate deeply into the RNA interior are from r-proteins L2 and L3, which approach the PTC as marked by the bound substrate analogue (Figure 10).

Our subsequent analyses of the structural features of the rRNA of the large subunit revealed a novel long-range RNA tertiary structure interaction, the A-minor motif, and a previously unrecognized secondary structure motif, the kink turn.[22,23] The A-minor motif involves the insertion of the smooth, minor groove (C2–N3) edges of adenine bases within single stranded regions into the minor grooves of neighboring helices, primarily at C–G base pairs. There are 186 adenines in the large subunit observed to make A-minor interactions that stabilize helix–helix, helix–loop, and junction interactions. Ramakrishnan et al. subsequently observed that A-minor interactions are important to decoding by stabilizing correct codon–anticodon interactions.[24] The kink-turns (K-turns) are

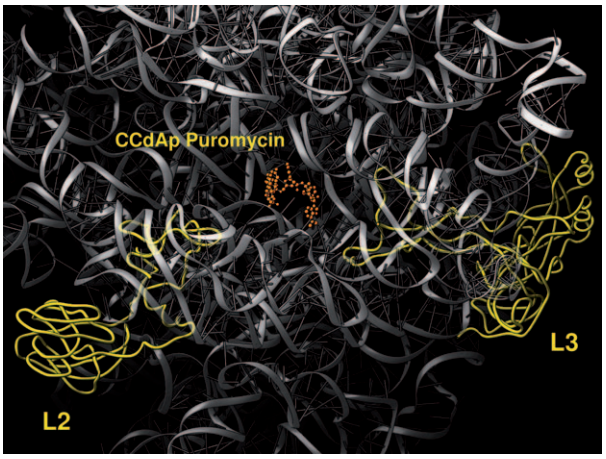

Figure 10. A ribbon representation of the 23S rRNA in white and proteins L2 and L3 in yellow showing the extended peptide chains penetrating into the ribosome interior towards the PTC but not reaching a bound substrate analogue (orange).

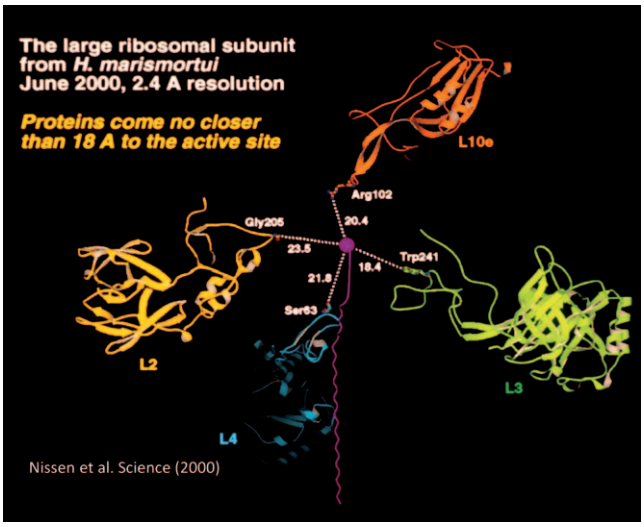

Figure 11. Four proteins whose non-globular extensions into the ribosome interior come the closest to the PTC (shown as a red ball), with distances to the PTC given in Angstrom.[14]

asymmetric internal loops imbedded in RNA double helices. The six K-turns in the Hma 50S subunit have a kink in the phosphodiester backbone that causes a sharp turn in the RNA helix, and they superimpose on each other with an rmsd of 1.7 Å.

Francis Crick had wondered in 1968 whether the catalytic heart of the ribosome was all RNA. Realizing that evolution had faced the "chicken or the egg problem" (which came first?) because the first machine to make a protein could not have been a protein, he wrote "*it is tempting to wonder if the first ribosome was made entirely of RNA*".[25] Noller and coworkers attempted to establish that indeed the ribosomal RNA is responsible for its catalytic activity by using proteases to digest the r-proteins.[26] However, many peptides in the 10000 molecular weight range, as well as intact L2 and L3, remained. Consequently, this experiment did not confirm the hypothesis that the catalysis is done by the RNA component of the ribosome.

When we examined the positions of all of the proteins that have portions that approach the heart of the PTC, we observed in 2000 that the closest protein component lies 18 Å from the PTC (Figure 11).[14] Even taking into account that a loop of protein L10e is disordered in this crystal and located in the neighborhood of the PTC, it cannot even hypothetically be extended into the PTC. Therefore, we were led to conclude in 2000 that "The ribosome is a ribozyme". This was the first experimental verification of the hypothesis that had been advocated by many in previous years.

The Mechanism of Peptide Bond Formation

As with any enzyme the important question is how catalysis is achieved, and in the case of the ribosome it is of particular interest how RNA can be effective in this process. Of course, as it the case with all enzymes, a major component, if not by far the largest contributor, is the enzyme's capacity to correctly orient the substrates in order that chemistry can occur.[27] This has been shown to be an important component also in ribosome catalysis (e.g., Sievers et al.[28]). But what other specific chemical mechanisms are utilized?

To address this question, many structures of the Hma large ribosomal subunit complexed with substrate, intermediate, and product analogues were determined by Jeff Hansen[14,29] initially and then subsequently by Martin Schmeing.[11,12] The reaction that is catalyzed is the attack of the α-amino group of the aminoacyl-tRNA bound in the A site on the carbonyl carbon of the peptidyl-tRNA bound in the P site. This leads to the formation of a tetrahedral carbon that contains an oxyanion; this intermediate then breaks down to form the product peptidyl-tRNA now in the A site and a deacylated P-site tRNA. Since it was not possible to bind full-length tRNA substrates to existing crystals of the 50S subunit, we made complexes with fragments of the 3′ end of tRNA containing either A, CA, or CCA linked to either the amino acid, peptide, or analogue of the tetrahedral intermediate. Biochemists had for many years used these kinds of substrate analogues to carry out what is called a "fragment assay" to study the reaction.

Initially, we determined the structures of substrate complexes with either CC-puromycin bound in the A site or CCA-Phe-caproic acid–biotin bound in the P site, which was stabilized in the P site by the simultaneous binding of sparsomycin.[29] To construct a model of the structure of a complex with aminoacyl-tRNA bound to the A site and peptidyl-tRNA bound to the P site, the structures of the complexes with the two substrate analogues were built onto the same model of the large subunit. The A- and P-site tRNAs from the Noller et al. model of the 70S complex with tRNAs[19] were also superimposed and joined to the fragment structures (Figure 12). As had been noted earlier,[14] the two tRNA molecules from residue 1 to residue 73 were related by a translation, while their CCA ends were related to each other by a 180° rotation. In the A site, C75 is Watson–Crick base paired to G2588 of the ribosomal A-loop, while in the P site

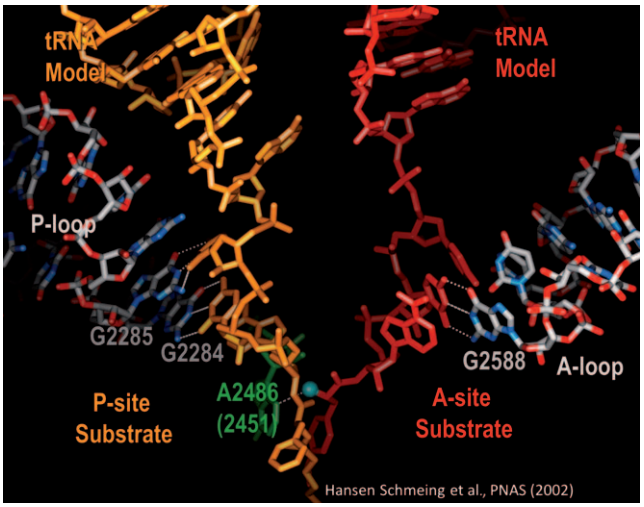

Figure 12. A model of the A-site and P-site substrates bound to the PTC constructed from the structures of the CCA-Phe-cap–bio bound to the P site (with sparsomycin, not shown) and C-puromycin bound to the A site, as well as models of the tRNA acceptor stems. The CCA of the P-site substrate makes two base pairs with G2285 and G2284 of the P loop, and C-puromycin makes one base pair with the A loop. The models of the acceptor stems of the A-site and P-site tRNAs are taken from the Yusopov et al.[19] model of the 70S ribosome with tRNAs bound to the A and P sites. The acceptor stems of the two tRNAs are related by a translation, while the two CCAs are related by a 180° rotation.

both C74 and C75 make Watson–Crick base pairs to G2285 and G2284 of the P loop. It was suggested[29] that the additional base pair between the CCA and the P loop in the P site as well as base stacking would increase the affinity of the CCA for the P site compared with the A site and thereby might facilitate the movement of the CCA and of the peptide linked A-site tRNA to the P site once the deacylated P-site tRNA had moved to the E site. These changes in the positions of the CCA ends of the tRNA may be responsible for formation of the hybrid state.

Martin Schmeing then determined the structures of many complexes of the large subunit with substrate analogues of A- and P-site substrates bound simultaneously to the PTC. Together, these suggested the mechanism of peptide bond formation and showed that the premature hydrolysis of the peptidyl-tRNA in the absence of an A-site substrate is suppressed by an induced fit mechanism.[12] To prepare a stable pre-reaction state complex, the A site substrate used was CC-hydroxypuromycin in which the α-amino group is replaced by a less reactive hydroxy group. In the absence of an A-site substrate, the ester linked carbonyl carbon of peptide linked to the P-site tRNA is protected from a nucleophilic attack by water on both sides by rRNA bases. Addition of the CC-hydroxypuromycin, however, causes a series of conformational changes in the rRNA that lead to the repositioning of the protective base and the reorientation of the carbonyl group positioning it for attack by the α-amino group. The structures of these complexes confirm that only the N3 of A2486 (2451 in *E. coli*) and the 2′-OH of A 76 of the P-site tRNA contact the attacking α-amino group of aminoacyl-tRNA and could be possible candidates for functioning as a general base to activate the nucleophilic attack of the α-amino group (Figure 13). Rachel Green and co-workers

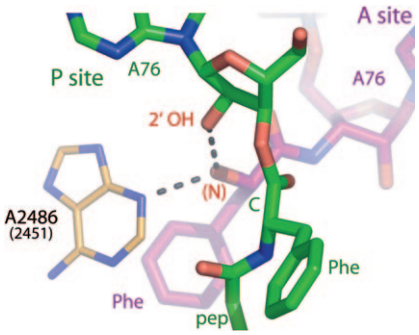

Figure 13. The pre-reaction ground state displaying the orientations of two fragment substrates bound to the PTC. The 2′-OH of A76 of the P-site substrate (in green) is H-bonded to the analogue of the α-amino group of the aminoacyl-CCA (in red). Only the 2′-OH of A76 and the N3 of A2486 (2451) interact with the attacking α-amino group.[12]

showed most conclusively that mutation of A2486 (2451) to any of the three other bases had no effect on the rate of peptide bond formation when full length substrates are used, thereby establishing that A2486 (2451) is not involved in catalyzing peptide bond formation.[30]

In contrast, removal of the 2′-OH of A76 of the P-site tRNA greatly reduces the rate of the peptidyl transferase reaction. Barta et al. found using fragment substrates that remove the 2′-OH of A76 of the P-site substrate reduced the rate of peptide bond formation by several hundred-fold.[31] Based on this observation and on structures of the Hma 50S subunit complexed with either a P-site substrate analogue or an A-site analogue, Barta proposed that the mechanism of peptide bond formation could be facilitated by a proton shuttle mechanism in which the 2′-OH of A76 acts as a general base to receive a proton from the α-amino group of the aminoacyl-tRNA to facilitate its nucleophilic attack while simultaneously acting as a general acid to provide a proton to the leaving 3′OH of the P-site A76 upon its deacylation. Strobel and colleagues demonstrated that if full tRNA substrates are used in these studies, then a 2′-deoxy-A76 in the peptidyl-tRNA resulted in a rate reduction in peptide bond formation of greater than 10^6-fold.[32] Thus, the 2′-OH of the P-site tRNA A76 is critical to peptide bond formation.

To explore whether the rate of peptide bond formation is also enhanced by stabilization of the tetrahedral transition state intermediate, Schmeing obtained a 2.3 Å resolution structure of a complex between the Hma 50S subunit and an analogue of the transition state that was synthesized by Kevin Huang in Scott Strobel's laboratory.[11,12] This analogue had a phosphate mimic of the tetrahedral carbon with an amino acid side chain mimic in place of one of the phosphate oxygens and a sulfur mimic of the oxyanion replacing the second oxygen. Hydrogen bonded to the phosphate oxygen mimic of the oxyanion is a water molecule that is positioned by two rRNA bases (Figure 14). This water molecule could indeed be

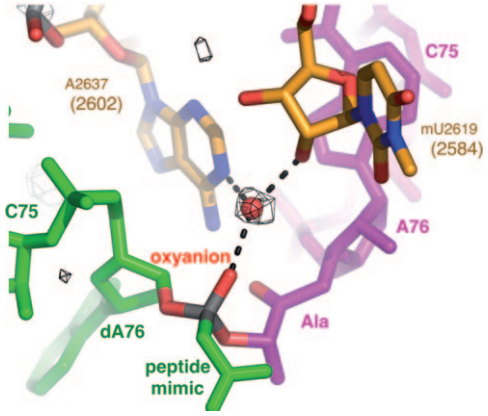

Figure 14. The oxyanion hole is a water molecule: Difference electron density in an F_o-F_c map (3.5 σ, 2.5 Å resolution) shows a presumed water molecule H-bonded to the oxyanion mimic of the transition state analogue, as well as to the N6 of A2637 (2502) and to the 2′-OH of mU2619 (2585).[11]

assisting in catalysis by partially compensating for the negative charge on the oxyanion.

Consequently, there appear to be at least three contributors to the ribosome's ability to enhance the rate of peptide bond formation. First, it correctly orients the two substrates. Second, it provides substrate assisted catalysis by the 2′-OH of A76 of the P-site tRNA that functions as a proton shuttle acting as both a general base and a general acid. Finally, a bound water molecule interacting with the oxyanion may be functioning to stabilize the transition state.

Antibiotic Inhibition of the 50S Ribosomal Subunit

About 50% of pharmaceutically useful antibiotics target the ribosome and the majority of these bind to the large ribosomal subunit. Our determination of the structure of the Hma large ribosomal subunit has enabled us to obtain the structures of its complexes with many families of antibiotics that bind in or near to the PTC, as well as those that bind in the E site.[29,33,34] Since *H. marismortui* is an archaeon, the antibiotic binding sites of its ribosomes are more similar to those of eukaryotic ribosomes than those of eubacterial ribosomes. Fortunately, at millimolar concentrations many antibiotics that target eubacteria will bind to the Hma large subunit, and our crystal structures of their complexes have enabled the structure-based design of more derivative compounds that are proving effective against resistant bacterial strains. Furthermore, complexes with Hma subunits that have been mutated to contain a eubacterial base bind these antibiotics at pharmacologically relevant concentrations and bind at a position that is displaced by less than an angstrom from that observed for the wild type Hma subunit. Consequently, these observations plus the high resolution of the structural studies that is possible with the Hma crystals have made the Hma large subunit structure a very effective tool in providing structural insights for the design of new antibiotics.

The macrolide family contains many members that have been pharmaceutically important over many years, e.g., erythromycin. The macrolides consist of 14- to 16-membered lactone rings to which various sugar substituents are attached. We were able to establish the structures of complexes with several 16-membered macrolides and one 15-membered macrolide bound to wild-type 50S subunit.[29] The macrolides bind just below the PTC in the polypeptide exit tunnel with the hydrophobic side of the macrolide ring stacking on two splayed-out bases that form a hydrophobic pocket. Although the oligosaccharide substitution on some macrolides, e.g., carbomycin A, overlap the substrate binding sites, most do not. They appear to be functioning by blocking the polypeptide exit tunnel thereby preventing the extension of the elongating polypeptide (Figure 15). I refer to this process as

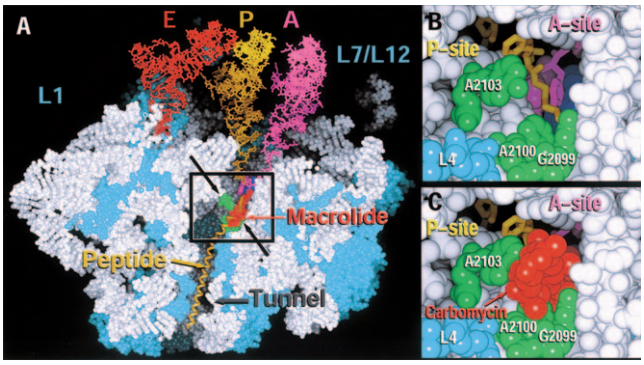

Figure 15. A) The structure of the macrolide carbomycin (red) bound to the 50S subunit, which is split in half with the 23S rRNA shown in white and the penetrating protein loops in blue.[29] The tRNA molecules are derived from combining the tRNA fragment structures complexed with the 50S subunit and the model of the tRNAs bound to the large subunit.[19] B) A view up the tunnel towards the PTC; the bases whose mutation render the ribosome resistant to inhibition by macrolides are shown in green. C) The same view as in (B) with the macrolide shown in red in a position that blocks the polypeptide exit.

"molecular constipation". Most of the macrolides interact only with the 23S rRNA and the positions of their macrolide rings superimpose on each other very well. Although there are almost no conformational changes induced in the RNA upon macrolide binding, the 16-membered macrolides cause a rotation of the base A 2103 (2100) and form a covalent bond with it.

Aligning the structure of the Hma subunit complexed with azithromycin with that of the *D. radiodurans* subunit bound to erythromycin[35] by superimposing their homologous rRNAs shows that the macrolide rings were positioned orthogonally in the two models which seemed surprising for two compounds that are chemically so similar.[29] Two possible explanations were posited for this difference initially. One possible cause might be the species differences; the second might be that the erythromycin was mis-positioned in the lower-resolution map (3.5 Å) of the Dra complex. Subsequent studies have established that the latter explanation is correct.[36]

Since the major difference between the eubacterial and archaeal binding sites for macrolides is residue A2058 in eubacteria, which is G2099 in archaea and eucaryotes,

mutation of A2058 to G in eubacteria reduces the affinity of the ribosomes for erythromycin by about 10^4-fold. Therefore, to better mimic the eubacterial ribosome, G2099 in the Hma 23S rRNA was mutated to an A.[36] While erythromycin does not bind to the Hma wild-type subunit at 1 mM concentration, it saturates the site of the mutant subunit at 0.001 mM concentration (Figure 16). Indeed, all antibiotics belonging

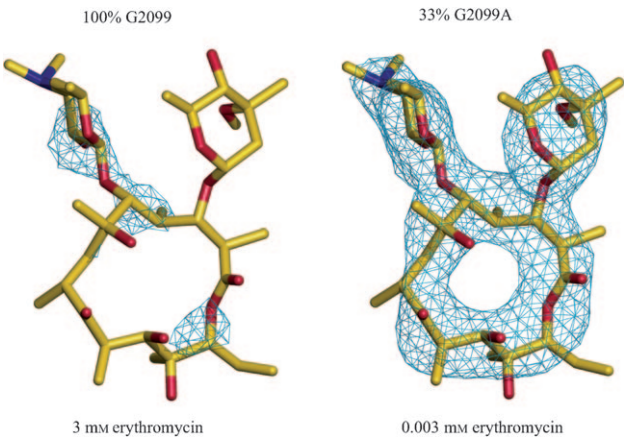

Figure 16. G2099A mutation increases erythromycin affinity >10000-fold: A difference electron density map between the wild-type 50S subunit containing G2099 soaked in 3 mM erythromycin and the apo 50S subunit (left) is compared to a difference map between a G2099A mutant 50S subunit soaked in 0.003 mM erythromycin and the apo-50S subunit (right).[36]

to the MLS_BK category that do not bind to the wild-type Hma subunit or to a eubacterial 50S subunit having an A2058G mutation bind to the G2099A-mutated Hma 50S subunit. Azithromycin likewise binds at a lower, more physiologically relevant concentration. Its orientation is the same as that observed in the complex with the wild-type 50S subunit, but it is positioned about 1 Å closer to the A2099 (2058) residue due to the lack of steric interference of the N2 of a G residue in that position. Very recently, we have determined the structure at 3.1 Å resolution of erythromycin bound to a 70S *Thermus thermophilus* ribosome and find that it binds identically as erythromycin binds to the mutated Hma 50S subunit.[37] This further confirms the earlier conclusion that the erythromycin was mis-oriented in the initial model of the Dra 50S subunit complex,[35] due presumably to the lower resolution of the electron density map into which the erythromycin was fit.

The structures of numerous other complexes between the Hma 50S subunit and different families of antibiotics that bind to the PTC have been determined.[33,36] Many bind to nearby, but distinct binding sites (Figure 17) and most inhibit protein synthesis by interfering with the binding of either the P-site or the A-site tRNA. The adjacent locations of these different antibiotic binding sites has provided the opportunity to create novel inhibitors by chemically tying a piece of one antibiotic to a piece of an adjacently bound one to create hybrid molecules that bind more tightly and provide the starting point for the creation of new antibiotics using computation and structure based design.

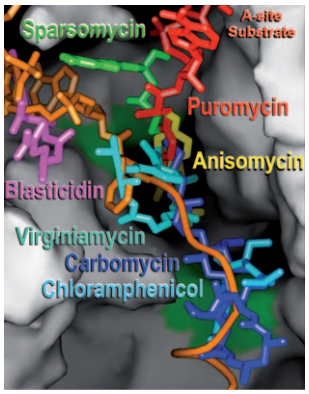

Figure 17. Seven different antibiotics are shown binding to adjacent but distinct binding sites in the PTC. The A site substrate is in red; the P site substrate with an extended peptide model is in orange.

Development of New Antibiotics by Rib-X Pharmaceuticals, Inc.

At a tRNA meeting held in Cambridge, England, in April of 2000, I had a fish and chips lunch with John Abelson at the Eagle Pub, where Francis Crick is reported to have first announced his and Jim Watson's discovery of the structure of double-stranded DNA and its significance for replication. John was a co-founder of Agouron Pharmaceuticals, which in the 1990's used a structure-based drug design approach to create one of the first HIV protease inhibitors; it became an approved pharmaceutical and has been used to successfully treat AIDS as part of combination drug therapy. I asked John if he thought we should start a biotech company to use our structural information on antibiotic complexes with the 50S subunit to design new antibiotic pharmaceuticals effective against resistant bacterial strains, and if so, would he be willing to participate in the founding of such a company? John very excitedly and enthusiastically said yes to both questions. We toasted the future and finished our meal while discussing strategies to explore.

In the following months we began to develop a plan. I asked Peter Moore to join in, which he did, and we decided that we should ask Bill Jorgensen to join the team because of his skills and accomplishments in computational methods of drug design. Susan Froshauer agreed to take on the role of CEO, which she has successfully done to the present time. After raising Angel funding mostly from friends, the company began in the summer of 2001 to use our structures of complexes between the Hma 50S subunit and antibiotics along with Bill Jorgensen's computational methods to carry out structure- and computation-based drug design. The company was named Rib-X Pharmaceuticals, to reflect the *rib*osome target and the use of *X*-ray crystallography to obtain structures. After eight years, their first drug candidate, radezolid, has successfully completed phase II clinical trials for use against skin and soft tissue infections and to treat mild-to-moderate community-acquired pneumonia. Other disease applications of radezolid are in phase II trials and a pipeline of additional compounds is nearing completion of preclinical trials.

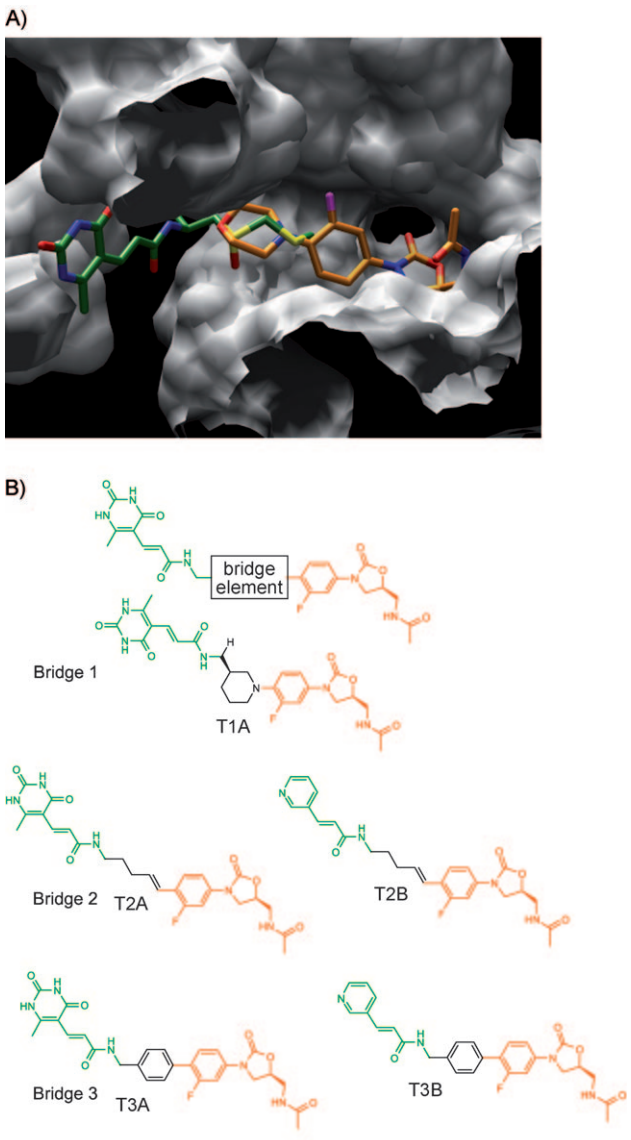

Figure 18. The creation of new hybrid antibiotic compounds by combining A) sparsomycin on the left (green) with linezolid on the right (orange). The ribosomal RNA to which they bind is shown in a surface representation (gray). B) These compounds can be chemically linked using various bridge elements to create hybrid compounds.

The design procedures used by Rib-X to ultimately obtain radezolid nicely exemplify how the structures of antibiotic complexes with the 50S subunit and computational methods can be effectively combined with pharmaceutical chemistry and microbiology to create new antibiotics that are effective against antibiotic resistant bacterial strains.[38,39] Linezolid, an antibiotic sold by Pfizer, binds to the PTC[34] adjacent to the binding site of the antibiotic sparsomycin,[33] which is not selective between eubacteria and eucaryotes (Figure 18a). In this example, portions of the two antibiotics are chemically linked together to create five new compounds whose intrinsic affinity, kingdom selectivity and minimum inhibitory concentrations (MIC) are measured (Figure 18b; Table 1). Two of the five were selective for eubacteria, showing that replacement of the key sparsomycin affinity element could alter the selectivity without completely losing ribosomal binding. Furthermore, the pair on the bottom (T3A and T3B) featuring the biaryl template showed not only substantially improved intrinsic affinity, but also dramatic improvement in antibacterial activity against representative community and nosocomial drug-resistant strains. With this proof-of-concept established, completely new molecules were designed. These took advantage of the ribosomal space defined by the chimeras, and they were optimized within these boundaries using computational methods to balance the molecular features so that Gram-positive and Gram-negative membranes could be penetrated, solubility and permeability could be maximized for oral bioavailability, and liabilities that might relate to toxicity were avoided. After synthesis of fewer than 700 compounds within less than one year's time, two drug candidates emerged: these featured greater than 10^3 lower inhibitory concentration for eubacteria than eucaryotes, very low MICs (0.25 and 2) against drug-resistant *S. pneumoniae* and *H. influenzae*, and oral efficacy in a variety of rodent models of infection. The final selected compound, radezolid, was found to be significantly more effective against many antibiotic resistant strains than the parent linezolid compound.

An analogous approach has led to the creation of a family of enhanced macrolides. This family features representatives of the 14-, 15-, and 16-membered macrolide families that have been augmented in novel ways to access adjacent, validated binding sites in the Hma 50S ribosome. By so doing, they not

Table 1: The minimum inhibitory concentrations (MICs) against three bacterial strains exhibited by five compounds created by chemically combining sparsomycin with linezolid.

	Linezolid (Lin)	Sparsomycin (Spa)	RX-154	RX-190	RX-209	RX-212	RX-213
		Intrinsic affinity (cell-free translation inhibition)					
E. coli D10 IC_{50} [μM]	4.6	<0.02	0.26	0.03	16	0.03	0.58
Bacterial selectivity	yes	no	no	no	yes	no	yes
		Antibacterial activity (MICs in μg mL^{-1})					
S. pneumoniae O2J1175 (Mac^R, efflux)	2	2	4	1	8	<0.25	0.5
S. pyogenes Msr610 (Mac^R, rRNA methylation)	1	2	4	1	4	<0.25	0.5
E. faecalis P5 (Lin^R, G2576U)	>32	>128	>128	32	>128	16	16

only restore activity against bacterial strains that are macrolide-resistant (e.g., the streptococci and the staphylococci, including community- and hospital-acquired MRSA), but also extend the spectrum to be effective against other drug-resistant Gram-positive bacteria such as the vancomycin-resistant enterococci. These compounds are in the late stages of preclinical testing.

Building on the knowledge that was derived from those programs, the Rib-X team undertook the de novo design of completely new antibiotics that target the 50S ribosomal subunit. Not only do they represent new classes for this important target, but also they have been optimized computationally to show potency against strains of multidrug-resistant Gram-negative organisms like *Escherichia coli*, *Psuedomonas aeruginosa*, and *Acinetobacter baumannii*. Additionally, because these compounds represent new chemical classes, they should not be affected by known resistance mechanisms seen clinically for other antibiotics. Thus, it appears that the structure of the Hma large ribosomal subunit and those of its complexes with antibiotics are enabling the development of a pipeline of new potential antibiotics.

New Antibiotics Against Tuberculosis?

Tuberculosis (TB) continues to be a major disease that causes over a million deaths a year, primarily in the poorest regions of the world. Also troubling is the recent emergence of strains, called XDR, that are resistant to all anti-TB antibiotics regardless of their specific molecular target. The possible spread of the XDR strains poses a potential medical problem for the rest of the world as well.

We have recently[40] determined the structures of the 70S *Thermus thermophilus* ribosome complexed with tRNA molecules bound to the A, P and E sites as well as capreomycin and viomycin, two tuberactinomycin cyclic peptide antibiotics effective against TB. They were known to bind only to the 70S ribosome and we observe them between the two subunits near the decoding center, interacting with tRNA and the β2A intersubunit bridge, which is formed by the contact between large subunit helix 69 and small subunit helix 44. The drugs interact with bases A1492 and A1493, stabilizing them in the "flipped out" orientation that they assume when assisting in mRNA decoding. It appears that the drugs stabilize the tRNA in the pre-translocation state (Figure 19).

Importantly, the capreomycin/viomycin binding site lies adjacent to the binding sites for two antibiotics that bind the small subunit, paromomycin[41] and hygromycin B[42] (Figure 20). This provides the opportunity to apply the same approach that Rib-X has been successfully employing to develop new anti-TB antibiotics by chemically tying a portion of either hygromycin B or paromomycin to capreomycin. Since the XDR strain may be the consequence of a mutation in an ion pump, a new, larger compound might prove effective.

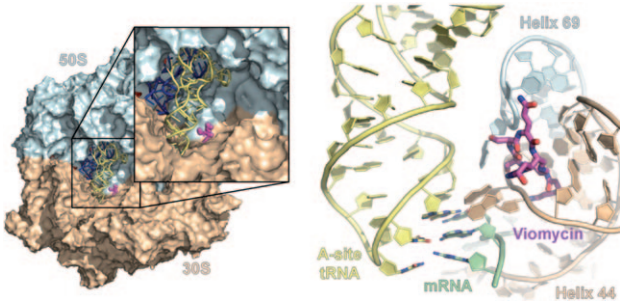

Figure 19. The binding site for viomycin at the decoding center interacting with RNA from both subunits.[40] Shown on the left is a surface rendition of the 70S ribosome with the 50S subunit in blue, the 30S subunit in tan, the A-site tRNA in yellow and viomycin in red. The close-up view shows viomycin bound to the large subunit helix 69 and small subunit helix 44 at the decoding center. Shown on the right are stabilizing bases 1492 and 1493 in their "flipped out" conformation, making A minor interactions with the codon of the mRNA (green) base-paired with the anticodon of the tRNA (yellow).

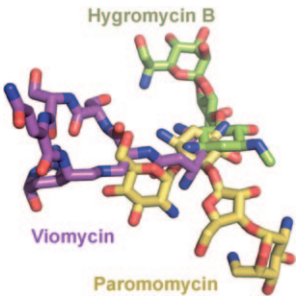

Figure 20. The adjacent binding sites of viomycin (purple), hygromycin B (green) and paromomycin (yellow) at the decoding center open the possibility of combinational drug design of new anti-TB antibiotics.[40]

Conclusion

We began our structural studies of the ribosomal large subunit in order to learn how this largest of RNA machines is built and how it is able to catalyze peptide bond formation. These basic science questions and answers have led to a practical and applied outcome that uses the power of structural and computational methods to design new potential antibiotics that are effective against antibiotic resistant bacterial strains. Our work reinforces my view of the importance of research funding agencies continuing to emphasize their support of basic research rather than divert their efforts to "translational" research, which I believe has a more limited horizon for novel discoveries.

I acknowledge the important contributions to the structural studies of the ribosome of all of the members of my research group as well as Peter Moore's group, during the past 15 years in addition to the four who are specifically mentioned in the text. I also wish to acknowledge the unique and enabling research environment created by the seven Center for Structural Biology (CSB) laboratories at Yale between 1995 to 2000 (Richards, Engelman, Moore, my lab, Sigler, Brünger, and

Doudna). Importantly, the long term and major support of my lab research and of the CSB by the Howard Hughes Medical Institute has been vital to the success of our studies of the ribosome. Support was also provided by a program project grant from the NIH. Finally, Erin Duffy assisted in the writing of the summary of drug development by Rib-X Pharmaceuticals, Inc.

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