BIOGRAPHICAL MEMOIRS

Alexander Rich

November 15, 1924–April 28, 2015
Elected to the NAS, 1970

A Biographical Memoir by Shuguang Zhang and Martin Egli

Alexander Rich was a biophysicist and a leader in the field of structural molecular biology. With his research team at MIT, he discovered the groundbreaking "left-handed" form of DNA, known as Z-DNA. He also made significant contributions to our understanding of the structural and functional relationships between RNA and DNA. In addition to his scientific work, Rich was a tireless advocate for world peace and nuclear disarmament.

Early Life and Education

Alexander Rich was born on November 15, 1924, in Hartford, Connecticut, to Jewish immigrants who fled persecution in Eastern Europe. His mother, Bella (Shub) Rich, of Horodetz, Belarus, immigrated to the United States with three teenage siblings in 1920 to join their father, Louis Shub, who had arrived six years earlier. The journey was delayed by war and the death of their mother, Esther, in the flu pandemic. Within a year, Bella married Max Rich, who had come from Ukraine, near Kiev, in 1913.

Although he grew up in Depression-era poverty in Springfield, Massachusetts, Alex said he never felt poor because his friends were all in similar situations. Alex's mother died when he was fourteen, and he lived with relatives during high school. At age seventeen, Alex was hired at the U.S. Armory. As the war effort surged, he worked full-time, third shift, while completing high school. With the ramp up of the war effort and his ability to run several machines concurrently, he was soon making more money than any of the parents in the neighborhood.

Figure 1 Alexander Rich holds a model of tRNA (transfer ribonucleic acid). *Photo courtesy of Donna Coveney, MIT.*

At the encouragement of his high school English teacher, he applied to Harvard College in 1942 and was awarded a tuition scholarship, which, as he would later recount, was in part because his last name did not sound Jewish, so he was not subject to the Jewish quota. Without the scholarship, his family could not have afforded for him to attend. He enlisted in the V-12 Navy College Training Program. Upon completion of the program in 1944, he was assigned to the hospital at the Portsmouth Naval Shipyard for six months, followed by medical training at Syracuse Medical School. Discharged

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from the Navy in 1946, he returned to Harvard College to complete his undergraduate degree. Returning to medicine at Harvard Medical School he worked with the biochemist John Edsall, who sparked his interest in the physical chemistry of biological macromolecules. He would also publish two scientific papers with Edsall. During this time, Rich had the opportunity to meet many of the era's leading scientists, including Cambridge University's John Desmond Bernal (a.k.a. the "Sage"), who was the mentor of several British Nobel laureates, including Francis Crick, Max Perutz, Dorothy Hodgkin, and Aaron Klug. Nearing completion of his M.D. degree, Edsall recommended Rich write to Linus Pauling at the California Institute of Technology (Caltech) to ask to join his group. Pauling was already legendary in chemistry, being the first to apply the principles of quantum mechanics, and was at the time working to determine models of protein secondary structure through X-ray diffraction analysis. By this time, Rich was hooked by the excitement of scientific discovery and left medicine to pursue science. He found out that an M.D. degree was sufficient to be a scientist, and often said "I don't accept Ph.D.s, I only give them out."

During this time, Rich not only was beginning to explore his intellectual possibilities, but also embarking on another long and happy personal journey. After completing his medical studies in 1949, he left for a trip to Europe on the trans-Atlantic ship RMS *Samaria,* where he met and courted his future wife, Jane King. They married and remained happily together for the next sixty-three years.

From Caltech to Cavendish (1949–mid 1950s)

In October 1949, Rich showed up in Linus Pauling's lab at Caltech in Pasadena, California, eager to learn. In those days, he made many lifelong friends among Pauling's team, including Martin Karplus, Matt Meselson, Gary Felsenfeld, David Davies, Carleton Gajdusek, Dick Marsh, Robert Shulman, and especially Jack Dunitz, who patiently taught Rich the art of X-ray crystallography. The small and intimate Caltech campus also meant that Rich mixed and became fast friends with many other scientific luminaries, such as Max Delbrück, Richard Feynman, James Watson, Benoit Mandelbrot, Leslie Orgel, Irwin Oppenheim, Verner Schomaker, Jerome Vinograd, and Hardin McConnell.

After working in Pauling's group for three years, Pauling suggested to Rich that he try to obtain X-ray patterns of DNA. At that time, because the camera was not optimal and X-ray radiation was very weak, it required a full day's exposure to collect the DNA fiber diffraction data. After a few months of painstaking work, Rich obtained well-oriented photographs. James Watson later remarked, at a conference celebrating the thirtieth anniversary of the discovery of the DNA double-helix in Cambridge, "Alex Rich arrived at Caltech and began to take X-ray photographs at just about the time that we proposed our model. I think it was inevitable that the structure would have been solved within about a year. The momentum was there, and they really knew DNA was important."

In September 1953, Pauling organized a conference on the structure of proteins and nucleic acids. In attendance was a group of British scientists that included Max Perutz, John Kendrew, and Francis Crick from Cavendish Laboratory in Cambridge and Maurice Wilkins and John Randall from King's College in London. It was a "Who's Who" in protein chemistry and molecular biology in its early days. During this conference, Crick invited Rich to Cambridge to use their rotating anode instrument for RNA diffraction, which deployed a more intense X-ray beam and required less fiber. In 1955, Rich traveled to Cavendish, supposedly for a three-week visit. Soon, however, after the arrival of Leslie Orgel and James Watson, intense discussions were underway about RNA structure and the daunting puzzle of the genetic code. Rich's initial short stay in Crick's house extended to over six months, and he was joined by Jane.

One morning, after Crick had read a *Nature* article over breakfast about a powder X-ray diagram and infrared spectrum of polyglycine II, Rich and Crick decided to build a skeletal brass molecular model of the protein polymer. By the afternoon, they had succeeded. During afternoon tea, Rich also realized that the unsolved structure of collagens had many glycine and proline or hydroxyproline residues, so the pair then started to build another model of repeating gly-pro-pro. The resulting model was written up in two landmark letters to *Nature* in 1955 and a full paper in the *Journal of Molecular Biology* in 1961. In 1995, forty years after their short letters, Helen Berman's lab reported the single-crystal structure of collagen. It turns out that this fits the Rich-Crick collagen model very well. We now know that twenty-eight types of collagens comprise ~30 percent of proteins in the human body. Collagen has been widely used for cosmetics and tissue repairs, and recombinant collagens have been produced in large scale. Because of his work on the structures of polyglycine II and collagen, Rich was recruited as a scientific advisor by DuPont in Wilmington, Delaware, a role he fulfilled for over a decade.

As Rich often remarked, in science, when you look back, everything is sunny and clear and some people even wonder why it took so long to make such discoveries; but when you look ahead, many things are in the fog and hard to navigate. After the DNA double helix structure was solved in outline through model building and low-resolution fiber X-ray diffraction in 1953, the questions that remained in the fog were the following: Would RNA form a double helix? Would DNA-RNA form a double helix? And how does DNA make

Figure 2 (l-r) Francis Crick, Alexander Rich, Leslie Orgel, and James Watson in Francis Crick's home in 1955. Please note their RNA Tie club ties, designed by George Gamow. *Photo courtesy of Alexander Rich's collection.*

RNA? In their short *Nature* letter on the DNA double helix, Watson and Crick specifically pointed out, "[It is] probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact."

National Institutes of Health (mid-1950s)

By 1952, Rich's fellowship from the U.S. National Research Council was coming to an end, and he was named head of the section on physical chemistry by Seymour Kety, who had just been appointed the first director of the newly formed National Institute of Mental Health at the U.S. National Institutes of Health (NIH) in Bethesda, Maryland. After arriving at the NIH in mid-1954, Rich then set about recruiting top researchers, including his Caltech colleague Jack Dunitz, and began a series of investigations using poly-riboadenylic acid (poly-rA) and poly-ribouridylic acid (poly-rU) to address these fundamental questions about RNA. In the spring of 1956, Rich started experiments with David Davies (another former Caltech colleague) mixing together poly-rA and poly-rU. To their astonishment, when the RNAs were mixed, they noted that the solution immediately became viscous. And when the resulting RNA fiber was analyzed by X-ray diffraction, the pattern was comparable to DNA. When Rich excitedly told one of his biochemist colleagues at NIH the results of his experiment, it was met with disbelief. "*Without an enzyme*?," his colleague asked incredulously. In the 1950s, it was inconceivable that two different complex biopolymers could combine into a single double helix without enzyme catalysis.

Rich had nevertheless discovered the first hybridization reaction, or "molecular sex," as British journalist Julian Huxley called it. It represented a paradigm shift in the way chemists and biochemists thought about interactions between macromolecular nucleic acids. Hybridization is the basis for all DNA-DNA, RNA-RNA, and DNA-RNA complementary recognitions and all the resulting technologies in the molecular biology toolbox, including DNA microarrays, antisense, RNAi, microRNA, PCR and CRISPR-Cas9. The DNA-RNA pairing also immediately suggests the genetic information transfer from DNA to RNA, namely transcription.

Massachusetts Institute of Technology (1958–2015)

In 1958, Rich accepted a position at the Massachusetts Institute of Technology (MIT) in Cambridge, Massachusetts, and worked there almost to his last day in life in April 2015 at more than ninety years of age. The Richs opened their house in Cambridge to the Crick family and a seemingly never-ending stream of scientists and interesting personages from around the world. After the discovery of mRNA, one big question in the following years was how RNA makes proteins. Deciphering the genetic code was underway thanks to the efforts of Marshall Nirenberg at the NIH using a cell-free system and poly-rU, poly r-AU and poly-rA. Crick had already determined each codon has three nucleotides by using bacterial phage genetics. And Crick proposed the adopter hypothesis, which turned out to be tRNA. But the question of how RNA made protein remained unclear.

Discovery of Polyribosomes

Rich asked his first graduate student, Jonathan Warner, to see if there were several ribosomes associated with each mRNA. They soon discovered that proteins are made not by single ribosomes, but by polyribosomes. Namely, each mRNA allows several ribosomes on it, like many train cars on a railroad track, to make proteins most efficiently and rapidly. In 1962, Rich submitted two papers, one reporting the electron microscopy studies, the other reporting the biochemical experiments using sucrose density gradient sedimentation and isotope tracing. After the first electron microscopy paper was published in *Science*, it was greeted with great skepticism,

until the second paper came out in the following month and several other labs confirmed their findings.

After the polyribosome discovery, Rich asked how the amino acids were joined inside the ribosome. Using rabbit reticulocyte cells, which have no nucleus and no longer synthesize RNA, they were able to do a rather clean analysis of the system by incubating the reticulocytes with radioactive adenine or cytosine. These nucleotides could penetrate into the interior, where they were incorporated into ribonucleoside-triphosphates. Although RNA was not synthesized, the 5′ CCA ends of tRNA molecules were continually cleaved off and then enzymatically re-attached to tRNA. The radioactive nucleotides thus labeled the ends of tRNA molecules and nothing else. This showed that two tRNA molecules were bound to active ribosomes, but only one was bound to inactive ribosomes at the top of the gradient. Rich and his colleagues postulated that the two tRNA-binding sites occupy adjacent codons. They called one "Site A," which bound aminoacyl-tRNA, and the other "Site P," which bound peptidyl-tRNA. Their idea was that these two sites acted in a coordinated manner to transfer the growing polypeptide chain and to move the mRNA codon from Site A to Site P. In addition, they suggested that it constituted the basis of ribosomal movement relative to the mRNA strand. When the crystal structure of the ribosome was finally determined thirty-seven years later, it was revealed that the ribosome indeed has an A site and a P site, as well as an exit "E site." Alex's lab also discovered the ~10 nanometer ribosome tunnel where the newly synthesized polypeptide chains exit the ribosome. This tunnel turns out to be the point where many antibiotics exert their action through "molecular constipation."

Determining the Structure of tRNA

After tRNA was identified in the1960s, efforts redoubled to attempt to obtain single crystals of tRNA; although methods for purifying tRNA continued to improve, good-quality tRNA crystals continued to remain elusive. In 1968, however, Sung-Hou Kim in the Rich lab was able to obtain single crystals of *Escherichia coli* $tRNA^{fMet}$. At the same time, three other groups also obtained single tRNA crystals diffraction but all with limited resolution. After numerous failures, in 1971, the Rich Lab reached an exciting turning point: Yeast $tRNA^{phe}$ could be crystallized in a simple orthorhombic unit cell with a resolution of 2.3 Å! These were the first crystals of tRNA suitable for detailed structural analysis. The key event in making these crystals was the incorporation of spermine, a naturally occurring polyamine. It was found later that the spermines were bound specifically to yeast $tRNA^{phe}$ and stabilized it so that it made a high-resolution crystal: an important innovation in crystal stabilization protocols at the time.

The L-shaped folding of the tRNA polynucleotide chain at 4Å resolution was a dramatic and surprising discovery, especially the separation between the acceptor site and the anti-codon. The backbone tracing was published on the front page of the *New York Times* on January 13, 1973, together with a discussion of its role in protein synthesis. No one had anticipated that the molecule would organize in this fashion. This folding was compatible with much of the biochemical experimental data concerning tRNA molecules. The final high-resolution structure was published in 1974 jointly with Sung-Hou Kim's group, who was a postdoc in the Rich lab and had moved to Duke University in 1972. The tRNA structure further confirmed Watson-Crick base pairing and also revealed numerous non-Watson-Crick base pairs and base triplets that, some twenty years later, were found in many ribozymes and in the RNA folds of ribosomes. The detailed tRNA structure and its complex interactions have stood the test of time.

Molecular Structures of the Double Helix

By the early 1970s, Rich's laboratory was turning its focus to the pursuit of high-resolution structures using single crystal X-ray diffraction. Single crystal growth was extremely difficult at that time; everything was done by trial and error, and each step took a very long time to accomplish. Finally, in 1973, John Rosenberg, Ned Seeman, and others in the Rich lab succeeded in determining the structure of r-GpC and r-ApU at 0.8Å resolution.

What did such a high-resolution structure mean for our understanding of nucleic acid base pairing? The GpC structure had the anticipated base pairs connected by three hydrogen bonds. But the ApU structure showed for the first time that Watson-Crick base pairs formed when the molecule was constrained in a double helix, as opposed to the Hoogsteen base pairs that were favored in the single-crystal complexes of adenine with uracil derivatives. Rich mailed preprints of the paper to several people, including Jim Watson, who soon telephoned Rich and said that the ApU manuscript had finally dispelled any uncertainty he had about the organization of the double helix, and that, after twenty years, he could finally get his first good night's sleep! The significance of the double helix at atomic resolution was recognized in a *Nature* "News and Views" commentary that called it the "missing link" and recognized that "the many pearls offered" helped resolve one of the big uncertainties in nucleic acid structure.

Discovery of Left-Handed Z-DNA

Rich was keenly aware that new methods and new technologies could accelerate scientific discoveries and change the way people do science. As soon as DNA oligonucleotide synthesis became available, he collaborated with Jacques Van

Boom, of the University of Leiden in the Netherlands, who synthesized and provided large quantities of oligo DNA d(C-GCGCG). Using this oligo DNA, Andy Wang in the Rich lab obtained crystals that diffracted to 0.9Å resolution, with every atom clearly visible. Heavy atoms were used to solve the structure, and they revealed, remarkably, a left-handed double helix with two antiparallel chains held together by Watson-Crick base pairs. Every other base had rotated around the glycosyl bonds so that the bases alternated in anti- and syn- conformations along the chain. The zigzag arrangement of the backbone Z-DNA was different from the smooth, continuous coil seen in B-DNA. The general response to this unusual structure was amazement coupled with skepticism. When Alex telephoned Francis Crick to report to him the unexpected finding of the left-handed double helix at 0.9Å resolution, there was a long silence at the other end of the telephone line. Alex then assured Crick that it might be a special case because it has a repeating d(CGCGCG) and some resulting special features.

The Z-DNA discovery generated excitement for physical and structural chemists and as well as structural biologists, but also skepticism for many biologists. It has been demonstrated that Z-DNA can be generated by negative supercoiling that is produced during DNA unwinding and gene transcription and recombination. Thus, Z-DNA requires higher energy to stabilize it, and its formation is transient and dynamic. It has also been found that many potential Z-DNA-forming sequences reside near the transcription start sites. Rich had a conviction that if there were alternative DNA conformations, nature would exploit them because evolution is opportunistic.

Elucidating a biological role for Z-DNA turned out to be a long and painstaking pursuit. Rich's lab, in collaboration with others, developed a Z-DNA-specific monoclonal antibody that was used to probe Z-DNA in the genome. These studies demonstrated that Z-DNA conformation often resides in transcriptionally active, rather than silent, genes, suggesting that Z-DNA has a biological role. Recent studies and reports by others have conclusively shown that Z-DNA indeed participates in many important biological regulations.

Polymath and Humanist

Alexander Rich not only was an extraordinary scientist who worked on extraordinary problems and who lived in extraordinary times, but he was also a very open-minded scientist, but above all, he was a humanist. His main contributions were to science, but he equally worked in the service of peace at a time when the threat of nuclear war was very real. His discoveries have made a major impact on our society, not only in generating new knowledge, but also in generating a new knowledge-based economy. It is believed this is one of

Figure 3 (l-r) Alexander Rich, Francis Crick, James Watson and Leslie Orgel at 40 year of DNA Double Helix. UNESCO, Paris, France. *Photo courtesy of Shuguang Zhang.*

the main reasons why his laboratory made so many important discoveries, often outside his principal research areas. His open-mindedness is captured by his most frequent comment: "*Why not*?" Alex inspired all of us in many ways. Alex's scientific curiosity never diminished, even increasing with age. Alex had an insatiable curiosity and was continuously fascinated by the latest discoveries. Nearing the end of his life, he was still eager to read the excitement of microbiome transplants, CRISPR, new synthetic organisms with six base pairs of DNA, and the expansion of the universe.

More than anything, one saw in Alex an original curiosity working. He was generous with his ideas and often stimulated others' imaginations. Some would say he carelessly gave away his ideas. One may wonder why he made so many important discoveries throughout his life. One explanation may be that his laboratory was always a microcosm of people from all backgrounds. He opened his laboratory not only to scientists, but also to artists, like Joe Davis. He invited people to his home for meals at a moment's notice. The parties on every occasion in his home were always memorable, gathering a great mix of interesting people. When asked why his laboratory made so many important discoveries, he summed it up in one phrase, "*Persistence is luck*." Truly, this was his mantra.

Alex was an integral part of the molecular biology revolution from its inception. He was one of twenty members of the historic RNA Tie Club, each member representing an amino acid. Alex was Arginine (for his initials A.R.) and served as the club's Lord Privy Seal of the British Cabinet. The RNA Tie Club was organized as a practical joke by physicist George Gamow (who also coined the term "Big Bang" of the Universe and proposed that the genetic code comprises a three-letter code). Gamow was Alanine (as the Ala) and

Jim Watson Proline (as the Pro). It included many physicists, including Richard Feynman (Gly), Edward Teller (Leu), Max Delbrück (Trp), Marvin Calvin (His), Francis Crick (Tyr), Sidney Brenner (Val), Leslie Orgel (Thr), Gunther Stent (Phe), and Erwin Chargaff (Lys), as well as biochemist Paul Doty (Asp). These names resonate with history.

Alex's service to science included a half century of support to the development of science in Israel. He held an advisory role with David Ben-Gurion in establishing the Weizmann Institute of Sciences in Israel during the 1950s. He traveled tirelessly to Israel almost annually, both alone and with his family, and served as a member of the Weizmann Institute's governing board from its inception until the end of his life.

Alex was a very active humanist, aiming to bring an end to the Cold War. During the peak of the Cold War, Rich traveled to the Soviet Union and befriended many leading Soviet scientists. Such interactions, though unpopular with many politicians at home, served to reduce tensions between the United States and the Soviet Union. With John Edsall, Linus Pauling, David Baltimore, and other leading scientists, he actively participated in the Pugwash Conferences on Science and World Affairs with leading scientists from the Soviet Union, China, and other countries. In London in 1962, a Russian colleague and Alex developed the idea that they might use automated seismographs to monitor nuclear testing—so-called black boxes. The proposal was then signed by several other American and Soviet scientists and became a document of the conference, which was subsequently sent to world leaders. At one stage, the Soviet Union contemplated using such automated seismographs, and it was one of the elements that facilitated the eventual signing of the Limited Test Ban Treaty in 1963. The conferences of the Pugwash movement, which was ultimately awarded the Nobel Peace Prize in 1995, served as useful forums for exchanging and discussing ideas and helped to minimize tensions then growing between the East and West. Although such conferences took an enormous amount of time, Alex considered his effort worthwhile because it addressed a major problem facing the world—the possibility of nuclear war.

Alex received many honors and awards, including the U.S. National Medal of Science, the Welch Award in Chemistry, and the Bower Award and Prize for Achievement in Science from the Franklin Institute, and the Lomonosov Gold Medal of the Russian Academy of Sciences. He was elected to all major scientific academies, including the Pontifical Academy of Sciences of the Vatican, where he helped to advise the Pope for decades, the French Academy of Sciences, the Russian Academy of Sciences, and the U.S. National Academy of Medicine. He received numerous honorary doctorates but none of these honors ever inflated his ego or affected his sense of purpose or his intense interest in the latest discoveries.

Rich was a great raconteur and enjoyed recounting the high suspense accompanying many of his scientific discoveries. He captivated his audiences from near and far with personal stories, sometimes scientific, but oftentimes not. He loved meeting people of all ages from all walks of life and collecting friends. His warm personality naturally attracted people to him. His sound and frank advice was widely sought out by people from all levels of social strata. He loved to take long walks with people and have animated talks. He often called a colleague and asked to take a walk during the weekend no matter where he was. In Cambridge or Woods Hole, everyone enjoyed walking and talking with him on all sorts of topics—anything that came to his mind, science, biology, chemistry, physics, astronomy, geology, medicine, clinical trials, technology, national and international politics, history, literature, a new idea he just had about new experiments, the latest film screen he just saw, the latest book or a scientific paper he just read, the latest seminar he just heard, fossil collections, and more. It was marvelous to take such walks with Alex.

If one can attempt to sum up Rich's personality in one word, it would be *open-mindedness*. His quality of open-mindedness not only opened the entire world for him, but also for everyone around him.

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