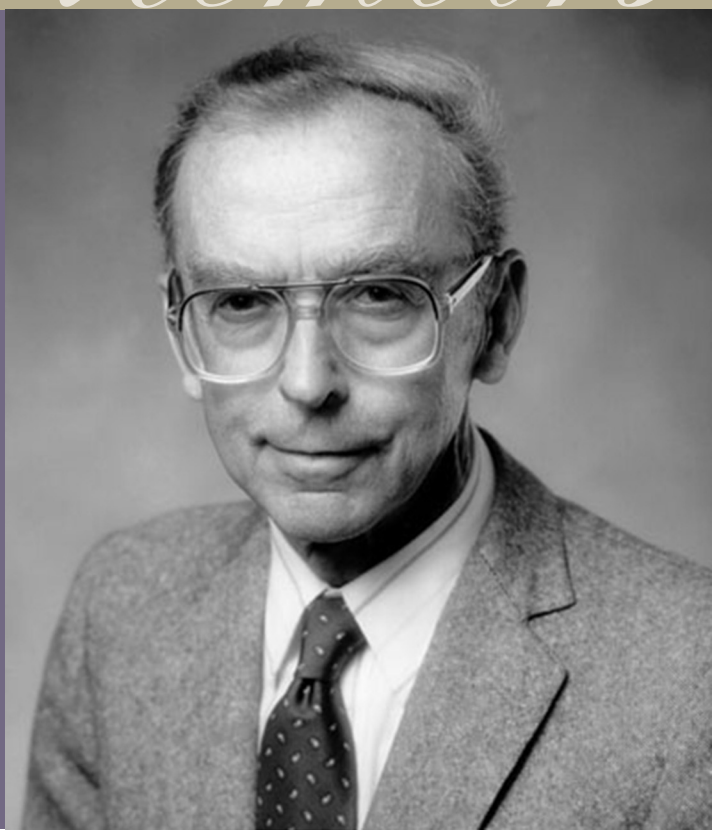
Robert W. Holley

1922–1993

BIOGRAPHICAL *Memoirs*



A Biographical Memoir by
Walter Eckhart

NATIONAL ACADEMY OF SCIENCES

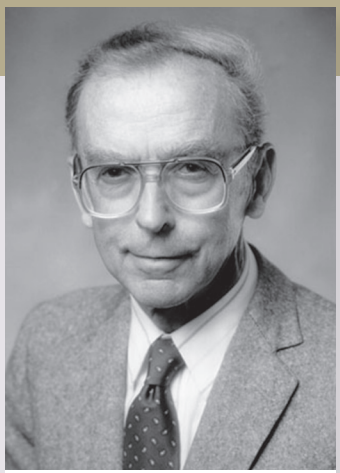
ROBERT WILLIAM **HOLLEY**

January 28, 1922–February 11, 1993

Elected to the NAS, 1968

Biochemist Robert W. Holley discovered the first nucleotide sequence of an RNA molecule, alanine transfer RNA (tRNA). This was also, indirectly, the first nucleotide sequence of a gene. For his groundbreaking research, Holley was awarded the Nobel Prize in Physiology or Medicine in 1968, sharing the honor with Har Gobind Khorana and Marshall Warren Nirenberg.

Reflecting on the nine-year duration of his work on alanine tRNA, Holley observed, "Without minimizing the pleasure of receiving awards and prizes, I think it is true that the greatest satisfaction for a scientist comes from carrying a major piece of research to a successful conclusion." At the close of his Nobel lecture, Holley remarked, "That then is our story of the alanine transfer RNA. It all followed quite naturally from taking a sabbatical leave. I strongly recommend sabbatical leaves."



Photograph courtesy of the Salk Institute

Robert Holley

By Walter Eckhart

Robert Holley was born in Urbana, Illinois, on January 28, 1922. He was one of four sons of Charles and Viola Holley, who were both educators. He graduated from Urbana High School in 1938 and then studied chemistry at the University of Illinois Urbana-Champaign, where he received hisbachelor's degree in 1942. He began his graduate work in organic chemistry at Cornell University, but his studies were interrupted in 1944 by World War II. He was moved to the research team of future Nobel Prize-winning biochemist Vincent du Vigneaud at Cornell Medical College, where he was involved in the first chemical synthesis of penicillin. He resumed his graduate work in 1946 and received his Ph.D. degree in organic chemistry in 1947.

After completing his Ph.D., Holley worked with Professor Carl M. Stevens at Washington State University as a postdoctoral fellow of the American Chemical Society (1947-–1948). He returned to Cornell as an Assistant Professor of Organic Chemistry at the university's Geneva Experimental Station in 1948. He was named associate professor there from 1950-1957.

During a sabbatical year (1955-56), he was a Guggenheim Memorial Fellow in the Division of Biology at the California Institute of Technology (Caltech), where he worked with molecular biologist James F. Bonner and first developed his interest in RNA. At the time it was becoming apparent that mechanisms of protein synthesis would be derived from a description of the components of in vitro protein synthesis systems. Studies had shown that amino acids were activated as amino acyl-adenylates by a family of enzymes. The next step was the attachment of amino acids to RNAs. The RNAs were referred to as soluble RNAs because they were found as supernatants after ultracentrifugation. They were later referred to as tRNAs.

At Caltech, Holley carried out experiments designed to detect the acceptors of activated amino acids by a different approach, the sensitivity of the acceptors to ribonuclease, the enzyme that degrades RNAs. Francis Crick had proposed the "adaptor hypothesis"—that a small nucleic acid molecule might act as an adaptor for aligning amino acids along an RNA chain during protein synthesis. It was apparent that the acceptor of activated amino acids was a low molecular weight RNA. For a chemist like Holley, the existence of amino acid-specific, low molecular weight RNAs was intriguing. He speculated that the RNAs might be small enough to permit structural studies. This would be of great interest because the nucleotide sequences of nucleic acids provide specificity and allow them to carry out their functions. For structural studies, a highly purified tRNA would be needed.

Renato Dulbecco, Roger Guillemin, Robert Holley, Francis Crick. (Courtesy of the Salk Institute.)

Holley returned to Cornell University in 1958 as a research chemist at the U.S. Plant, Soil and Nutrition Laboratory of the U.S. Department of Agriculture on the Cornell campus. (The university now hosts the Robert W. Holley Center for Agriculture & Health, named in his honor.) He held an appointment at Cornell during this time and was named Professor of Biochemistry in 1962. He rejoined the Cornell faculty full time in 1964 as Professor of Biochemistry and Molecular Biology and served as chair of the department from 1965 to 1966.

Upon his return to Cornell, Holley set out to isolate an individual tRNA for chemical study. The first step in the project required purification of tRNAs. Holley developed the technique of countercurrent distribution into a generally applicable method for the fractionation of tRNAs. He selected yeast tRNA because it could be obtained in large quantities. Isolation of alanine tRNA was an enormous task that eventually required four years and about 200 grams of bulk yeast tRNA obtained by phenol extraction of about 140 kilograms of commercial baker's yeast yielding one gram of highly purified material.

Even after purification, it was not clear that the material was sufficiently pure to proceed with structural analysis. Several attempts to fractionate the material further were unsuccessful, so Holley decided to proceed. He later observed, "There seemed no alternative but to gamble a few years of work on the problem, hoping that the material was sufficiently pure for structural analysis."

Establishment of the polynucleotide sequence of the alanine tRNA took another three years. The experimental approach Holley used was similar to one used earlier by Fred Sanger to discover the amino acid sequence of insulin, namely, to cut the molecule into fragments and assemble the fragments in the correct order, based on overlaps between fragments.

Holley cut the polynucleotide chain of alanine tRNA with two different enzymes: pancreatic ribonuclease, which cleaves after pyrimidine nucleotides, and takadiastase ribonuclease T1, which cleaves the RNA chain at guanylic acid residues. The fragments were separated by chromatography on DEAE cellulose, a recently designed ion-exchange chromatographic technique. The isolated fragments were analyzed further by progressive degradation from one end, using an exonuclease, snake venom phosphodiesterase, and assembled in order based on overlaps between the fragments. The analysis was made more difficult and time-consuming by the presence of modified nucleotides in the RNA, which had unusual properties. (The modifications were introduced after the tRNA was transcribed from DNA.) The final sequence consisted of 77 nucleotide residues.

The sequence led to speculation about the folding of the tRNA molecule that would expose the sequence of the anticodon. The "cloverleaf" secondary structure of the tRNA assumed that there would be Watson-Crick-type pairing of A to U and G to C in the double-stranded regions, and that the unpaired regions would form loops. The triplet sequence that constitutes the anticodon in the alanine tRNA was found in the middle of the molecule and was situated in such a way as to allow it to interact with a triplet of nucleotides (the codon) in the mRNA. The nucleotide sequences of tRNAs found since

then also fit the cloverleaf model and have their anticodons situated in a similar position.

Holley's colleague Elizabeth Keller used the tRNA sequence to figure out the cloverleaf structure of the alanine tRNA molecule. According to Cornell legend, it is said that she sent the model to Holley in a Christmas card.

The sequence was completed in 1964. Its importance in explaining the synthesis of proteins from mRNA was quickly recognized. Several structures of tRNAs were determined within the following two years.

Holley spent 1966–67 as a National Science Foundation Postdoctoral Fellow at the Salk Institute for Biological Studies and the Scripps Clinic and Research Foundation in La Jolla, California. Holley would be awarded the Nobel Prize in Physiology or Medicine in 1968, together with Har Gobind Khorana and Marshall W. Nirenberg, for their contributions to the understanding of the genetic code and protein synthesis. That same year, he joined the permanent staff of the Salk Institute as a Resident Fellow and American Cancer Society Professor of Molecular Biology. He was also an adjunct professor at the University of California, San Diego.

After joining the Salk Institute, Holley turned his attention to factors that control the growth of mammalian cells. These factors are important in normal processes such as wound healing and in diseases such as cancer. After some experiments on mouse 3T3 cells, he focused on BSC-1 cells, an epithelial cell line, because most human cancers are epithelial in origin.

Robert Holley at the Salk Institute. (Courtesy of the Salk Institute.)

Holley resisted the idea of "contact inhibition" of cell growth in culture, caused by physical contact between adjacent cells, rather favoring the idea that the inhibition was a consequence of a reduced supply of nutrients. He recognized the role of polypeptide hormone-like materials, and eventually isolated inhibitors of epithelial cell growth.

In addition to directing research, Holley continued to work actively in the laboratory on his own experiments, somewhat unusual for a senior scientist. He remained at the Salk Institute until his death, at age 71, in 1993.

Robert Holley at the microscope. (Courtesy of the Salk Institute.)

Holley was a warm and generous mentor who treated the members of his laboratory with affection and compassion. He exerted a positive influence because of his character, integrity, and enthusiasm for science.

Holley received many awards and recognitions for his scientific work. He was a member of the National Academy of Sciences (elected in 1968), the American Academy of Arts and Sciences, and the American Association for the Advancement of Science. He was a member of the American Society of Biological Chemists and the American Chemical Society. In addition to the Nobel Prize, he received the Albert Lasker Award in Basic Medical Research in 1965, the Distinguished Service Award of the U.S. Department of Agriculture in 1965, and the U.S. Steel Foundation Award in Molecular Biology of the National Academy of Sciences in 1967.

Robert Holley married Ann Dworkin, a mathematics teacher, in 1945. He and Ann enjoyed walking near the beach in La Jolla and visiting the nearby mountains. They had one son, Frederick, who became a physician. He graduated from the Stanford University School of Medicine in 1979 and went on to specialize in anesthesiology.

Although strongly dedicated to his scientific work, Holley had interests outside the laboratory, notably sculpture. He molded small models out of clay and then converted them to large, elegant bronze pieces. He was particularly fond of modeling dancers. Many of his sculptures were constructed in his study at the Salk Institute. He might have had a quite different career, had he elected not to go into science.

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