Insights into DNA Joining: I. Robert Lehman’s Work on DNA Ligase

Deoxyribonucleic Acid Ligase. Isolation and Physical Characterization of the Homogeneous Enzyme from Escherichia coli

Deoxyribonucleic Acid Ligase. A Steady State Kinetic Analysis of the Reaction Catalyzed by the Enzyme from Escherichia coli

I. Robert Lehman was born in Tauragė, Lithuania in 1924. He and his family immigrated to the United States in 1927 and settled in Baltimore, Maryland. During the summer of 1943, less than 3 months after graduating from high school, Lehman was drafted into the army and served in the infantry in France and Germany. Upon his return to the United States he took advantage of the GI Bill of Rights and attended college at the government's expense. He majored in chemistry at Johns Hopkins with the intention of becoming an industrial chemist, like an uncle that he admired. However, a biology course taken during Lehman’s senior year changed his plans. The main emphasis of the course was biochemistry, and Lehman became fascinated by the pathways of carbohydrate, lipid, and energy metabolism. He abandoned his plans to become an organic chemist and, following his graduation in 1950, he applied to the Doctoral Program in the Department of Biochemistry at the Johns Hopkins School of Public Health. Lehman was accepted into the program and joined Roger Herriott’s laboratory. His doctoral dissertation was on the metabolic changes that occurred in Escherichia coli following infection with phage T2 and the “ghosts” of T2, which were formed when the DNA was released from the phage by osmotic shock.

After receiving his Ph.D. in 1954, Lehman decided to pursue research in intermediary metabolism. He recalls, “I was convinced, after completing my doctorate, that the most excitement in biochemistry was in intermediary and energy metabolism. This was reinforced by a talk that I heard given by Irving Lieberman, then a postdoctoral fellow with Arthur Kornberg, at the 1954 Federation Meeting in Atlantic City, on the discovery of phosphoribosyl pyrophosphate and its role in pyrimidine nucleotide biosynthesis” (1).

As a result, Lehman joined Alvin Nason at the McCollum-Pratt Institute at Johns Hopkins University. Nason was doing work on the role of metal ions in electron transport in Neurospora, and Lehman was given the task of identifying a metal cofactor for a particulate NAD-linked cytochrome c reductase. However, this postdoctoral fellowship proved to be short-lived, as Lehman explains. “Although I approached the project with considerable enthusiasm, I soon decided that it was not for me. Lieberman’s 10-minute Federation talk was still very much on my mind and I went to the library and read every paper Kornberg had published on coenzyme and nucleotide biosynthesis that I could find. I then wrote Arthur asking if I could join his lab as a postdoctoral fellow. To my great good fortune, he accepted me” (1).

Lehman arrived at Washington University in St. Louis during the summer of 1955. During the next 3 years he and other members of the Kornberg laboratory discovered the first DNA polymerase, DNA polymerase I from E. coli, and showed that it was a template-directed enzyme. This work, which eventually earned Kornberg one-half of the 1959 Nobel Prize in Physiology or Medicine, is the subject of a previous Journal of Biological Chemistry (JBC) Classic (2).
In 1958, Lehman began independent research as an Instructor in the Department of Microbiology at Washington University. Looking for a project, he noted the importance of specific proteases in the analysis of protein structure and sequence and was struck by the absence of comparable enzymes that acted on DNA. Using *E. coli*, Lehman began to search for the DNA enzymes and was able to purify exonuclease I.

In the summer of 1958, Kornberg was offered the Chair of Biochemistry at the Stanford University School of Medicine, and he invited the Microbiology faculty at Washington University to join him in forming the new Biochemistry Department at Stanford. Lehman accepted, and his new laboratory was up and running in the summer of 1959. Lehman continued his search for DNases in *E. coli* and other organisms and purified several of these enzymes.

In the late 1960s, Lehman started looking for a new area of research. He heard a seminar given by Matthew Meselson, in which he described his work with Jean Weigle, demonstrating that genetic recombination occurred by the breakage and rejoining of the recombining DNA molecules. Lehman decided to look for the enzymes that catalyzed the joining of DNA molecules. As he was purifying the polynucleotide joining enzyme, he became aware that several other laboratories were working on the same problem in a variety of organisms. Each group had its own name for the enzyme, but they all settled on “DNA ligase,” and friendly competition between the labs ensued. Lehman and his graduate student Paul Modrich were able to work out the mechanism of the joining reaction, which is the subject of the two JBC Classics reprinted here.

The first Classic describes Lehman and Modrich’s purification of DNA ligase from *E. coli*, as well as some of its physical properties. In the second Classic they report on the kinetic analysis of the reaction catalyzed by the pure enzyme as well as several of its partial reactions. These studies, coupled with previous findings of others in the Lehman lab including Baldomero Olivera, Zach Hall, and Richard Gumport, showed that the formation of the phosphodiester bond proceeds through a pathway involving the generation of two intermediates. First, ATP reacts with DNA ligase to form a ligase-AMP intermediate in which the adenylyl group is bound by a phosphoamide bond to a lysine in the active site of the enzyme. Then, the activated adenylyl group is transferred to the phosphate group at the 5’ terminus of a DNA chain, forming a DNA-adenylate complex. In the final step there is an attack of the 3’-hydroxyl of the DNA on the activated 5’-phosphoryl group to form a phosphodiester bond. It was later discovered that this mechanism also held for the T4 DNA ligase and for the mammalian ligases.

Subsequent studies of *E. coli* and T4 mutants by Lehman and several others showed that DNA ligase is essential for the joining of Okazaki fragments during DNA replication, the joining of DNA chains during nucleotide and base excision repair of DNA, and the joining of DNA segments following cleavage of the Holiday junction in homologous recombination.

In addition to his work on ligase, nuclease, and polymerase, Lehman also described recombinational strand transfer by RecA protein recombinase and established key features of the
mechanism of herpes. He was named William Hume Professor at Stanford University in 1980 and continues in this position today. He served as Chairman of the Department of Biochemistry from 1974 to 1979 and from 1984 to 1986. In recognition of his scientific achievements, Lehman received the American Society for Biochemistry and Molecular Biology (ASBMB) Merck Award in 1995. He is a member of the American Academy of Arts and Sciences (1973) and the National Academy of Sciences (1977) and holds honorary doctorates from the University of Gothenberg and the University of Paris. Lehman was President of ASBMB from 1997 to 1998, was on the Editorial Board of the Journal of Biological Chemistry from 1969 to 1974, and has served as an Associate Editor for the journal since 1990.\(^1\)

Lehman’s coauthor on the Classics, Paul Modrich, is currently James B. Duke Professor in the biochemistry department at Duke University Medical Center, where he studies DNA mismatch repair. He will be the subject of an upcoming JBC Classic.

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REFERENCES


\(^1\) Biographical information on I. Robert Lehman was taken from Refs. 1 and 3.
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