*Protein Science* (1998), 7:1061–1066. Cambridge University Press. Printed in the USA.


**RECOLLECTIONS**

# Recollections of a DNA enzymologist

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(RECEIVED December 22, 1997; ACCEPTED January 22, 1998)

*Early years in Baltimore*

Like many of my generation, my life was profoundly influenced by World War II. I was drafted into the army, at 18, in the summer of 1943, within two months of my high school graduation. I served in the infantry in France and Germany, a wrenching experience that only those who have faced enemy fire in ground combat can really comprehend. It left a deep impression which I carry with me to this day. But World War II was also responsible for my becoming a scientist. The GI Bill of Rights, an inspired federal program, permitted me, like millions of other returning veterans, to attend college. It paid my full tuition and even provided a $50 per month stipend.

I majored in chemistry at Johns Hopkins with the ambition of becoming an industrial chemist. I thoroughly enjoyed organic chemistry and I was good at it. However, in my senior year I took a biology course given by Bill McElroy who was the Director of the newly formed McCollum-Pratt Institute. The main emphasis was on biochemistry and, in particular, intermediary metabolism. I found it tremendously exciting. It was precisely the kind of chemistry that I wanted to do.

The war years were still very much with me and I was unwilling to leave my family in Baltimore, so I decided to remain at Johns Hopkins for graduate work. My doctoral thesis advisor was Roger Herriott, a protein chemist who, before coming to Hopkins, had been associated with John Northrop and Moses Kunitz, two eminent protein chemists at the Rockefeller Institute. He had turned from protein chemistry to bacteriophages, a field which, having lain dormant for many years, was expanding rapidly with the work of Max Delbruck, Alfred Hershey, Salvador Luria, Seymour Benzer, Seymour Cohen, and others in the newly formed phage group.

My doctoral dissertation involved a study of 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. There were indeed major changes in oxygen uptake and $CO_2$ release that probably reflected changes in glycolysis and the pentose phosphate pathway. There were also changes in phosphate metabolism, all of which were duly recorded in my thesis. However, I failed to detect the profound alterations in nucleotide metabolism that were needed for the synthesis of T2 phage DNA, in particular the hydroxymethylcytosine, that replaced the cytosine in T2, T4, and T6 bacteriophages.

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. I still recall my awe and excitement at these revelations.

Still reluctant to leave Baltimore, I applied to Albert Lehninger, chairman of the Department of Physiological Chemistry at Johns Hopkins Medical School, and a leading light in oxidative phosphorylation, for a postdoctoral position. He did not accept me, with an excuse that his lab was full. I next approached Nathan Kaplan at the McCollum-Pratt Institute. Here again, I was turned down; however, his lab was indeed full. His crowded quarters in an old greenhouse on the Hopkins campus could not possibly accommodate even one more person. Nate did suggest that I talk to Alvin Nason, also at McCollum-Pratt, who was doing interesting work on the role of metal ions, notably molybdenum, in electron transport in *Neurospora*. Al accepted me into his lab and I began work on a particulate NAD-linked cytochrome *c* reductase, with the aim of identifying a metal cofactor.

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

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**I. Robert Lehman** received his undergraduate degree in chemistry in 1950, and his Ph.D. degree in biochemistry in 1954, both at the Johns Hopkins University. After a one-year postdoctoral period at the McCollum Pratt Institute of Johns Hopkins University, he spent three years as a postdoctoral fellow in the Department of Microbiology at Washington University in St. Louis, then joined the faculty as an Instructor. In 1959 he became a member of the newly formed Department of Biochemistry at Stanford University and has remained there. He served as Chairman from 1974–1979 and 1984–1986. He is a member of the National Academy of Sciences and the American Academy of Arts and Sciences. He holds honorary doctorates from the University of Gothenburg and the University of Paris. He is a recipient of the ASBMB Merck Award, and is currently President of the American Society for Biochemistry and Molecular Biology.

good fortune, he accepted me. We have remained colleagues and close friends ever since.

*DNA polymerase in St. Louis*

I arrived in St. Louis in the summer of 1955, a summer notable for having broken a long-standing record for the number of consecutive days in which the temperature reached or exceeded 100 °F. Arthur was chairman of the Department of Microbiology at Washington University, whose faculty, in addition to himself, consisted of Paul Berg, Melvin Cohn, Robert De Mars, David Hogness, Irving Lieberman, and Philip Varney. Later, Dale Kaiser replaced De Mars, who had left to discharge his military obligation with service at the NIH. Shortly thereafter, Jerard Hurwitz joined the faculty. The Department was housed in rather dilapidated quarters in the top floor of the old Washington University Clinic Building. The elevator was reputed to be the oldest still in operation in the city of St. Louis and riding it could often be an adventure. There was a journal club that met daily at lunch. All the members of the department participated and we were joined by Martin Kamen who was in the Department of Radiology, and Stanley Cohen who was working on Nerve Growth Factor in Rita Levi-Montalcini's lab on the main campus. The discussions were spirited, critical, often combative, and to me, somewhat intimidating. But they were great fun. Arthur's group consisted of Uriel Littauer, a postdoc from Israel; Jose Fernandez, a postdoc from Brazil; his late wife Sylvy Kornberg; and Ernie Simms, a technician.

The discovery that the DNA of the T-even phages contained hydroxymethylcytosine in place of cytosine seemed terribly important, and I was eager to learn how hydroxymethylcytosine was made. Arthur felt that this was a good project and managed to acquire a small amount of [$^{14}$C] $\beta$-carbon labeled serine, a presumed donor of the hydroxymethyl group, for my experiments. Within a short time I found that extracts of T4-infected *E. coli* did incorporate the $^{14}$C label into dCMP, and began fractionation to purify the enzyme. At about the same time, Arthur had observed that small amounts of $^{14}$C-labeled thymidine that he had obtained from Morris Friedkin in the Pharmacology Department were incorporated into an acid-insoluble product by *E. coli* extracts. The counts were low but they were made acid-soluble upon treatment with pancreatic DNase. I recall his telling me of this tantalizing finding and I must confess that I was somewhat envious. When he invited me to put the hydroxymethyl dCMP experiments on hold and join him, I jumped at the chance. Shortly thereafter, we learned that Seymour Cohen had discovered the T4 phage dCMP hydroxymethylase and had opened up the whole field of virus-induced enzymes. But I have never regretted my decision.

Arthur has described in several places, most notably in his autobiography "For the Love of Enzymes," the trail of research that led from the incorporation of a few counts of [$^{14}$C]-thymidine into DNA by *E. coli* extracts, to the discovery of the enzyme that we named DNA polymerase; the discovery of the four deoxynucleoside triphosphates that were its substrates; the recognition that all four triphosphates as well as a DNA primer and template were needed; and finally, the stunning realization that these requirements were all a reflection of the fact that the polymerase was faithfully copying the DNA template, something that was unprecedented in biochemistry. Arthur's group, which initially consisted of Simms, Sylvy Kornberg, and me, was joined by Maurice Bessman, Julius Adler, and Steven Zimmerman, Arthur's first graduate student. Howard Schachman, who was a sabbatical visitor at the time, taught us about ultracentrifuges and viscometers and demonstrated that the product of the DNA polymerase reaction was indeed macromolecular.

I view those days on the fourth floor of the old Clinic Building at Washington University to be among the most thrilling and enjoyable of my scientific career. There were new and unexpected findings being made all the time, and we all shared in the joy and excitement of discovery.

In the summer of 1957, I received an offer of an assistant professorship at McCollum-Pratt from Bill McElroy. I had been a postdoc for two years, and under normal circumstances, it would have been time to move on. Moreover, academic positions were hard to come by. However, it was clear that I was working a gold mine, and I had no intention of leaving. I continued working as a postdoc for an additional year and then began independent research as an Instructor in the department.

*Nucleases*

In looking around for a project, I had been impressed by 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. Our purification of DNA polymerase from *E. coli* extracts and constant attempts to rid the enzyme of nucleases that degraded the DNA product suggested that *E. coli* might be a good source of such enzymes. The first of these that I purified, exonuclease I, turned out to be extraordinarily useful. It was absolutely specific for single-stranded DNA and was therefore very effective, in fact the only reagent at the time that could distinguish single-stranded from double-stranded DNA. A notable example of its usefulness was in the early DNA renaturation studies of Paul Doty, Julius Marmur, and Carl Schildkraut. After heat or alkaline denaturation of rather heterogeneous preparations of calf thymus or salmon sperm DNA, renaturation yielded duplex DNA with single-stranded tails. These produced rather messy, often uninterpretable, banding patterns in the CsCl density gradients that were used to measure renaturation. However, upon treatment with exonuclease I, to remove the single-stranded tails, sharp bands appeared, making it clear that true renaturation had occurred. Exonuclease I was also instrumental in Robert Sinsheimer's discovery that the single-stranded DNA of phage $\theta$X174 was circular. Although single-stranded, it was degraded by exonuclease I only after being nicked by an endonuclease. Another fortunate property of exonuclease I that I was able to exploit was its ability, in contrast to all other known nucleases, to degrade glucosylated (T-even phage) DNA completely to mononucleotides. It had been found some years before that the T-even phage DNAs contained glucose linked to the hydroxymethyl cytosine that replaced cytosine. Ann Pratt and I were able to determine the patterns of glucosylation of the hydroxymethylcytosine residues, and discovered to our amazement that in T4, in which all of the hydroxymethylcytosines are glucosylated, half contained glucose in the $\alpha$ configuration, and the other half in the $\beta$ configuration. Later, Sylvy and Arthur Kornberg discovered that T4 actually encodes distinct $\alpha$ and $\beta$ glucosyl transferases. In phage T6 DNA, the disaccharide gentiobiose was the predominant glycosyl residue. This was all very exciting, and I was convinced that these elaborate patterns of glycosylation must be terribly important. It now appears that their only function is to protect the phage DNA from restriction.

In the summer of 1958, Arthur was offered the chair of Biochemistry at the Stanford University School of Medicine and in-

vited the Microbiology faculty, including me, its most junior member, and Robert (Buzz) Baldwin, then at the University of Wisconsin, to join him in forming the new Biochemistry Department at Stanford. I had never been west of St. Louis, but the opportunity was too good to pass up. Again, Arthur has described the move West in his autobiography, and it need not be repeated here. Suffice it to say that within several weeks of our arrival at Stanford in the summer of 1959, the labs were up and running.

Buoyed by our success with exonuclease I, Stuart Linn, a graduate student, Ian Kerr, a postdoc, and I continued our search for DNases in *E. coli* and branched out to other microorganisms. We purified several of these enzymes; all were specific for single-stranded DNA, and showed preferential cleavage of certain sequences, but none were truly base- or sequence-specific. We missed the jackpot—the restriction endonucleases. Stuart Linn did, however, in his subsequent postdoctoral work with Werner Arber in Geneva, discover the first restriction endonuclease, EcoB.

In the mid-1960's I was invited by Bob Sinsheimer to present a seminar at Caltech. During my visit I met with Bob Edgar who told me of his work with *amber* and temperature-sensitive mutants of phage T4 that were defective in DNA replication. As a consequence of work in the Cohen, Kornberg, and Bessman labs, it was clear that the T phages induced the synthesis of a variety of enzymes required for the replication of their DNA, including a novel DNA polymerase. Edgar offered to send me a series of DNA negative T4 mutants, in the hope that we would be able to determine which was defective in the phage DNA polymerase. Adrian de Waard, a Dutch postdoc, and a graduate student, Aniko Vessey Paul, quickly demonstrated that extracts prepared from cells infected by *amber* mutants in gene 43 were lacking in the T4 polymerase. The same discovery was made almost simultaneously by Huber Warner at the University of Minnesota. We then went on to show that temperature-sensitive gene 43 mutants produced a temperature-sensitive DNA polymerase, thus demonstrating that gene 43 was indeed the structural gene for the T4 DNA polymerase. This was an important result because it demonstrated for the first time that the DNA polymerase activity that we and others were measuring in vitro is required for DNA synthesis in vivo. The role of T4 DNA polymerase in T4 DNA replication in vivo was further reinforced by experiments carried out by Zach Hall, a temporarily transplanted neurobiologist, who showed that the DNA polymerase purified from cells infected with T4 mutator mutants in gene 43 was mutagenic in vitro. These results became all the more important when DeLucia and Cairns, several years later, in 1969, found a mutant of *E. coli* lacking DNA polymerase (i.e., DNA polymerase I) and questioned the role of DNA polymerase in DNA replication. More about that later.

### *Ligase*

While casting about for a new area of research in the late 1960's, I heard a seminar given by Matthew Meselson, in which he described his work with Jean Weigle, which demonstrated quite clearly that genetic recombination occurred by the breakage and rejoining of the recombining DNA molecules and not by a replication mechanism in which portions of the two molecules were copied alternately by a DNA polymerase (Meselson referred to this as copy-choice). What were the enzymes that were able to catalyze the joining of DNA molecules? Baldomero (Toto) Olivera had joined the lab after training in DNA physical chemistry with Norman Davidson at Caltech and I suggested to him that we look for an enzyme that could promote such a joining reaction. The substrate that we devised was a poly (dA) chain of about 1000 nucleotides to which were annealed multiple 100 nucleotide-long poly (dT) segments, each labeled with $^{32}P$ at its 5′ terminus. This, in effect, produced a duplex DNA molecule with nicks spaced at 100 nucleotide intervals. Joining was measured by conversion of the $^{32}P$-labeled 5′ phosphomonoester to a phosphodiester with *E. coli* alkaline phosphatase which could hydrolyze the monoester but not the diester. The very first experiment demonstrated a joining activity in our *E. coli* extracts.

Early on, we noted that increasing amounts of extract did not produce a corresponding increase in activity. At low levels of extract there was virtually no joining activity. The reactions were performed in the presence of ATP and increasing the level of ATP did not eliminate the lag. Something was limiting. On the assumption that the limiting factor was a heat-stable cofactor, we added a small amount of "kochsaft," a boiled extract of *E. coli*, a classic maneuver that dates back to the days of Meyerhof and Embden and the resolution of the glycolytic pathway. The lag disappeared. This, of course, provided us with an assay with which to purify the cofactor. It turned out to be NAD, the pyrophosphate bond of which was cleaved to produce AMP and nicotinamide mononucleotide, an extraordinary and unprecedented use of a redox coenzyme.

As we were purifying the polynucleotide joining activity, we became aware that a similar activity had been discovered in four other labs. Martin Gellert at the NIH had found it in *E. coli* extracts, Charles Richardson and Bernard Weiss at Harvard, Jerard Hurwitz and Malcolm Gefter at Albert Einstein, and Arthur Kornberg and Nicholas Cozzarelli, my next door neighbors at Stanford, had all found it in extracts of T4 infected cells. The phage enzyme used ATP rather than NAD. Each group had its own name for the enzyme (Hurwitz's "sealase" was the most colorful) but we all settled on DNA ligase, the term coined by Richardson.

There was an exciting period of intense, but generally friendly, competition among the labs studying the enzyme. My group, Toto Olivera, Zach Hall, Paul Modrich, and Richard Gumport, were able to work out the mechanism of the joining reaction, which involved formation of a ligase-AMP intermediate in which the adenylyl group of NAD is bound by a phosphoamide bond to a lysine in the active site of the enzyme, releasing nicotinamide mononucleotide. Then the adenylyl group is transferred to the 5′ phosphate group of the DNA and linked by a pyrophosphate bond. 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 with the release of AMP. The mechanism for the *E. coli* enzyme also held for the T4 DNA ligase and, in fact, for the mammalian ligases of which there are as many as four now known. The use of NAD as a cofactor seems to be confined to bacteria; the T4 phage and mammalian ligases all use ATP instead of NAD.

Subsequent studies of *E. coli* and T4 mutants by several labs, including my own, showed that DNA ligase is essential in vivo for the joining of Okazaki fragments during DNA replication, the joining of DNA chains during nucleotide and base excision repair of DNA, and in the joining of DNA segments following cleavage of the Holiday junction in homologous recombination. And, of course, DNA ligase became a key reagent in the construction of recombinant DNA molecules. I don't believe that any of us working on this enzyme foresaw the central role it would play in the genetic engineering revolution. That was left to Paul Berg, Peter Lobban, Stan Cohen, and Herb Boyer.

*Back to DNA polymerase*

The DeLucia-Cairns mutant, announced in *Nature*, was a bombshell. Although lacking DNA polymerase activity, the mutant was fully viable; its only defect was an increased sensitivity to ultraviolet irradiation. Now our DNA polymerase was relegated to the lowly role of a "repair enzyme." Some different system was thought by many to be responsible for chromosomal replication, possibly using substrates other than the deoxynucleoside triphosphates. Ironically, with the realization that many human cancers result from DNA repair defects, DNA repair is currently regarded as one of the hottest fields in biology.

Although Cairns was right in believing that our DNA polymerase (Pol I) was not responsible for chromosomal replication, he was wrong in evaluating its role. With *E. coli* extracts apparently deficient in Pol I, DNA polymerase II, and subsequently DNA polymerase III holoenzyme, each present in relatively few copies per cell, could be detected and isolated. With polymerase III identified as part of a machine responsible for replication of the *E. coli* chromosome, how did Pol I fit into the picture?

I had been deeply distressed by the Cairns paper and all of the ensuing publicity. I was determined to see if the Cairns mutant was really lacking in Pol I. It was a risky project, one that I felt best to work on myself. With the help of my technician, Janice Chien, I was able to show that the extracts of the *pol A*$^-$ strain, which bore an *amber* mutation, did contain a low level of Pol I (1–2% of wild type), resulting from readthrough of the *amber* codon. However, the levels of the $5' \rightarrow 3'$ exonuclease activity associated with Pol I were normal. The $5' \rightarrow 3'$ exonuclease polypeptide was, however, far smaller than the intact enzyme. Pol I contains two domains: one with polymerase activity associated with $3' \rightarrow 5'$ proofreading exonuclease and a second containing a $5' \rightarrow 3'$ exonuclease. Protease treatment of Pol I yields the large or Klenow fragment with polymerase and $3' \rightarrow 5'$ exonuclease activities and a small fragment with the $5' \rightarrow 3'$ exonuclease. It turned out that the *amber* mutation in the Cairns mutant was positioned in such a way that translation yielded the intact small fragment, explaining our observation that the mutant extracts contained normal levels of $5' \rightarrow 3'$ exonuclease activity, of a size corresponding to that of the small fragment.

Bruce Konrad and I subsequently isolated a temperature sensitive mutant defective in the $5' \rightarrow 3'$ exonuclease activity but normal in its polymerase activity. Under standard growth conditions, the $5' \rightarrow 3'$ exonuclease of Pol I is essential for DNA replication. The Cairns mutant was viable because it retained this activity. We now know that an essential role of Pol I in DNA replication is the $5' \rightarrow 3'$ exonuclease removal of the RNA primers that initiate Okazaki fragment synthesis, prior to their being joined by DNA ligase.

Robert Bambara and Dennis Uyemura purified Pol I from several of pol A mutants with defects in the polymerase domain. Because their $5' \rightarrow 3'$ exonuclease activity was unchanged, they were fully viable; however, they were abnormally sensitive to UV radiation. In examining various features of the polymerase activity in these mutants, Bambara and Uyemura developed what I believe to be the first quantitative way to assess the processivity of deoxynucleotide polymerization by DNA polymerases. High processivity is of course essential for chromosome replication and we now know that there are complex protein assemblies that interact with DNA polymerases to tether them to the template, in order to prevent their dissociation during deoxynucleotide polymerization.

As another extension of our work on DNA polymerase mutants, Duane Eichler, a postdoc, and then later Per Olaf Nyman, a sabbatical visitor from Sweden, and Bik Kwoon Tye, a postdoc, examined the formation and processing of Okazaki fragments in these mutants. Their joining was clearly retarded to an extent that depended on the severity of the defect. We could support the claims of Reiji and Tuneko Okazaki that their pulse-labeled fragments were intermediates in DNA replication (on the lagging strand) rather than artifacts of DNA strand scissions, a possibility that had not yet been excluded.

*Eukaryotic DNA replication*

At about this time, I began to think about eukaryotic DNA replication. An obvious place to start was with a DNA polymerase. Two nuclear DNA polymerases had been described: DNA polymerase $\alpha$ (Pol $\alpha$) was believed to be involved in chromosomal replication and DNA polymerase $\beta$ (Pol $\beta$) in DNA repair. The field was highly populated and rather contentious. Pol $\alpha$ seemed to come in various sizes and there was no agreement about its molecular weight and number of subunits. Its cellular abundance is low, so that barely microgram quantities emerged from the rather elaborate purification procedures that had been devised.

In casting about for an abundant source of Pol $\alpha$, I decided on *Drosophila melanogaster*. David Hogness, in our Department, had shown earlier that chromosomal replication in early stage embryos of *Drosophila* proceeded at a frenetic pace: the entire *Drosophila* genome was replicated in about three minutes. This was a consequence of the large number of replication forks operating in tandem. It struck me that such embryo extracts must be highly enriched in DNA polymerase (presumably Pol $\alpha$). Assays of the extracts showed that this was indeed the case.

Our purification was begun by two sabbatical visitors, the late John Boezi, from Michigan State, and Geoffrey Banks, from Mill Hill in London. The project then passed on to Laurie Kaguni, Guiseppi Villani, and Brian Sauer. They showed that Pol $\alpha$ consisted of four subunits, of which the largest, at 180 kilodaltons, contained the DNA polymerase activity. Ron Conaway, a graduate student, then demonstrated that the most purified preparations of Pol $\alpha$ contained primase activity (primase had not yet been demonstrated in eukaryotes); he and Laurie Kaguni showed that the primase was associated with the two smaller of the four subunits. The association of primase with Pol $\alpha$ in *Drosophila* was quickly confirmed by several laboratories in eukaryotes as diverse as baker's yeast and humans.

*Rec A*

While this work on Pol $\alpha$ was in progress, we had actually begun a completely new line of investigation on genetic recombination in *E. coli*. Kevin McEntee, who joined my lab as a postdoc, had, as part of his doctoral research at the University of Chicago, constructed a specialized lambda transducing phage, containing the *rec A* gene. The *rec A* gene had been identified by John Clark in the early 1960's as essential for homologous recombination in *E. coli*, but its product had never been isolated, and no one had any idea of what it did. Kevin's postdoctoral fellowship application had involved a study of some of our Pol I mutant enzymes. But on deciding on a project, when he arrived in the lab, the idea of isolating the *rec A* gene product and determining its function seemed

far more attractive. George Weinstock, who arrived at about the same time, joined forces with McEntee.

The *rec A* gene product had been known to be involved in radiation-induced mutagenesis or error-prone repair as it was known. We therefore devised an assay for its purification that looked for nucleotide misincorporation during replication of single-stranded θX174 DNA in vitro. After nearly a year of failure, we decided, in desperation, to try something heretical. Induction of Kevin's transducing phage yielded large amounts of the Rec A protein, easily visible at the predicted size of 40 kilodaltons in an SDS-polyacrylamide gel of the induced crude extracts. The idea was simply to purify the 40 kilodalton polypeptide without a functional assay. The danger, of course, was that we would end up with a pure but totally inactive protein. This was a particularly risky strategy with a protein of unknown function. Unconventional then, this has now become almost standard procedure with cloned and over-expressed gene products.

With pure Rec A protein in hand, we were in a position to test it for various activities that are known to be associated with enzymes that act on DNA: DNA and RNA polymerase, nuclease, DNA-dependent ATPase, etc. However, before we could perform any of these assays, we heard a presentation by Tomoko Ogawa, of the University of Osaka, at the 1978 Cold Spring Harbor Meeting, in which she showed that a purified preparation of the Rec A protein had ATPase activity dependent on single-stranded DNA. At about the same time, Jeffrey Roberts and Nancy Craig, who had been studying the regulatory role of the *rec A* gene and identified the Rec A protein as a protease, also discovered its DNA-dependent ATPase activity. McEntee quickly confirmed the Ogawa's and Craig and Robert's findings with our Rec-A protein preparation. He then went on to demonstrate that the DNA-dependent ATPase associated with the Rec A protein purified from a cold-sensitive *rec A* mutant was also cold sensitive. This result was important because it ruled out contamination of Rec A protein preparations with one or more of the many DNA-dependent ATPases that are present in *E. coli*. Coincident with these studies, George Weinstock was investigating the fate of the single-stranded DNA during the ATP hydrolysis and found that complementary single strands were being renatured. McEntee and Weinstock then quickly showed that not only could the Rec A protein promote the renaturation of complementary single strands, but it could also promote ATP-driven insertion of a single strand into an homologous DNA duplex, to form a D-loop. In essence, the Rec A protein could form a Holiday junction, the key intermediate in homologous recombination, thereby explaining the essentially of the *rec A* gene. Findings similar to ours were made at about the same time by Charles Radding and by Steve West and Paul Howard-Flanders at Yale. Again, there was an intense, but largely friendly, competition between the various groups.

The Rec A group, which initially consisted of McEntee and Weinstock, quickly grew once we published our findings. Michael Cox, Peter Riddles, Randy Bryant, Zvi Livneh, Douglas Julin, and Daniel Soltis joined the lab in the next five years and contributed to our attempts to understand the mechanism by which the Rec A protein promotes strand exchange, and several, notably Cox and Bryant, have continued to work productively on the Rec A protein after leaving my lab.

The nine year period that we worked on the Rec A protein was exciting, productive, and ultimately very gratifying. However, important features of Rec-A-promoted strand exchange still remain elusive. For example, a key step in the process, the mechanism by which the Rec A protein searches for and finds the homologous regions between recombining DNA molecules, is still a mystery. Nevertheless, we did go a long way toward solving a fundamental problem in biology. There may also be broader consequences of this work. The human Rec A analogue, the Rad51 protein, seems to play an important role in embryogenesis: embryos of Rad51 "knockout" mice survive for only a few days, and Rad51 has recently been shown to interact with BRCA1 and BRCA2, the breast cancer susceptibility gene products.

During this period, we continued to work on eukaryotic DNA replication. Although our expectation that *Drosophila* embryos would be an abundant source of replication enzymes proved correct, we were unsuccessful, despite the rapid rate of DNA replication in those embryos, in finding extracts that could promote origin-dependent DNA replication. Part of the problem was the uncertainty about a *Drosophila* origin; no origin had been unequivocally identified. At the same time, several labs, notably those of Tom Kelly, Jerry Hurwitz, and Bruce Stillman had demonstrated SV40 origin-dependent DNA replication in mammalian cell extracts and were well on their way to identifying and resolving the components that were involved. Was there another viral chromosome with a defined origin that we might explore?

### *Herpes virus DNA replication*

Edward Mocarski, from the Microbiology Department at Stanford, had informed us of the herpes simplex type 1 virus (HSV-1) and its life cycle. It contained not one but three identifiable origins of replication. But it was rather large and unwieldy (152 kilobases). Nevertheless, introduction of one of these origins into a 5-kilobase plasmid permitted its replication in HSV-1 infected cells. Replication generated long concatomers, indicating that it proceeded by a rolling circle mechanism.

In recent years we have tried to reconstitute HSV-1 DNA replication with purified enzymes. Unlike SV40, HSV-1 encodes most of the enzymes it needs to replicate its genome, including a DNA polymerase, a single-strand DNA binding protein, a polymerase processivity factor, a primosome (helicase-primase) and an origin-binding protein. The last three were discovered in my lab. Again, I was fortunate to have been able to recruit a splendid group of postdocs and students: Per Elias and Mike O'Donnell started the project, and were joined later by James Crute, Robert Bruckner, Mark Dodson, Rami Skaliter, and Sam S-K Lee as postdocs, and Tom Hernandez, Rebecca Dutch, and Boris Zemelman, as graduate students. We are enormously aided in our efforts by Ed Mocarski, a seemingly inexhaustible source of information and ideas about herpes viruses. Although we have succeeded in reconstituting rolling circle replication, the initial, origin-dependent phase of replication still eludes us and remains a challenge for the future.

Perhaps this account should have been entitled "Wanderings of a DNA Enzymologist." However, this wandering has not been entirely aimless. If there is a unifying theme to my research, it is the search for and study of enzymes that alter DNA molecules: polymerases, nucleases, ligases, recombinases. Although I would certainly not have predicted it, I am still in pursuit of these wonderful enzymes, more than forty years after I started. They have never lost their fascination for me nor do I expect that they ever will.

## References


Banks GR, Boezi JA, Lehman IR. 1979. A high molecular weight DNA polymerase from *Drosophila melanogaster* embryos. *J Biol Chem 254:*9886–9892.

Conaway RC, Lehman IR. 1982. A DNA primase activity associated with DNA polymerase $\alpha$ from *Drosophila melanogaster*. *Proc Natl Acad Sci USA 79:*2523–2527.

Crute JJ, Tsurumi T, Zhu L, Weller SK, Olivo PD, Challberg MD, Mocarski ES, Lehman IR. 1989. Herpes simplex virus 1 helicase-primase: A complex of three herpes-encoded gene products. *Proc Natl Acad Sci USA 86:*2186–2189.

Elias P, Lehman IR. 1988. Interaction of origin binding protein with an origin of replication of herpes simplex virus 1. *Proc Natl Acad Sci USA 85:*2959–2963.

Konrad EB, Lehman IR. 1974. A conditional lethal mutant of *Escherichia coli*. K12 Defective in the 5′ $\rightarrow$3′ exonuclease associated with DNA polymerase I. *Proc Natl Acad Sci USA 71:*2048–2051.

Lehman IR. 1960. The deoxyribonucleases of *E. coli*. I. Purification and properties of a phosphodiesterase. *J Biol Chem 235:*1479–1487.

Lehman IR, Bessman MJ, Simms ES, Kornberg A. 1958. Enzymatic synthesis of deoxyribonucleic acid. I. Preparation of substrates and partial purification of an enzyme from *E. coli*. *J Biol Chem 233:*163–170.

Lehman IR, Chien JR. 1973. Persistence of DNA polymerase I and its 5′ $\rightarrow$3′ exonuclease activity in PolA mutants of *Escherichia coli* K12. *J Biol Chem 248:*7717–7723.

Lehman IR, Herriott RM. 1958. The protein coats or "ghosts" of coliphage T2. III. Metabolic studies of *E. coli* B infected with T2 bacteriophage "ghosts." *J Gen Physiol 41:*1067–1082.

Lehman IR, Linn S, Richardson CC. 1965. Nucleases as reagents in the study of nucleic acid structure. *Federation Proc (Symposia) 24:*1466–1472.

Lehman IR, Pratt EA. 1960. On the structure of the glucosylated hydroxymethylcytosine nucleotides of coliphages T2, T4 and T6. *J Biol Chem 235:*3254–3259.

Lehman IR, Zimmerman SB, Adler J, Bessman MJ, Simms ES, Kornberg A. 1958. Enzymatic synthesis of deoxyribonucleic acid. V. Chemical composition of enzymatically synthesized DNA. *Proc Natl Acad Sci Wash 44:*1191–1196.

McEntee K, Weinstock GM, Lehman IR. 1979. Initiation of general recombination catalyzed by the RecA protein of *E. coli*. *Proc Natl Acad Sci USA 76:*2615–2619.

Olivera BM, Lehman IR. 1967a. Linkage of polynucleotides through phosphodiester bonds by an enzyme from *Escherichia coli*. *Proc Natl Acad Sci Wash 57:*1426–1433.

Olivera BM, Lehman IR. 1967b. Diphosphopyridine nucleotide: A cofactor for the polynucleotide-joining enzyme from *Escherichia coli*. *Proc Natl Acad Sci Wash 57:*1700–1704.

Skaliter R, Makhov AM, Griffith JD, Lehman IR. 1996. Rolling circle DNA replication by extracts of herpes simplex virus type 1-infected human cells. *J Virol 70:*1132–1136.

Uyemura D, Bambara R, Lehman IR. 1975. On the processive mechanism of *Escherichia coli* DNA polymerase I. *J Biol Chem 250:*8577–8584.