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# NEVER A DULL ENZYME

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## GROWING UP WITHOUT SCIENCE (1918-1942)

The current generation of scientists may be suprised to know that I had no formal research training. I was well started in a career of clinical medicine until World War II placed me in the National Institutes of Health (NIH) where I soon became an eager investigator of rat nutrition. Three years later, in 1945, I responded to the lure of enzymes and have remained faithful to them ever since.

Science was unknown in my family and circle of friends. Once, in 1947,

when I was in the biochemistry department of Washington University in St. Louis, working under the guidance of Carl and Gerty Cori, Gerty told me that Carl had collected beetles and butterflies in his youth, and then asked: "Arthur, what did you collect?" "Matchbook covers," was my sheepish response. What else? They were the dominant flora in the Brooklyn streets where I played and in the subways where my father often risked being trampled when he stooped to add one more to my collection.

My early education in grade school and Abraham Lincoln High School in Brooklyn was distinguished only by "skipping" a few grades and finishing three years ahead of schedule. I recall nothing inspirational from teachers or courses except encouragement to get good marks. I remember the glow of my chemistry teacher when I received a grade of 100 in New York State Regents examination. It was the first time, in more than twenty years of teaching, a student of his had gotten a perfect grade. Once when I boasted about this to my wife, Sylvy, she remarked that she too had gotten 100, not only in chemistry, but also in algebra and geometry.

I chose the cachet of City College in uptown Manhattan over nearby Brooklyn College, even though commuting from Bath Beach (near Coney Island) meant three hours a day in crowded subways. Competition among a large body of bright and highly motivated students was fierce in all subjects. I carried over my high school interest in chemistry, but the prospects for employment in college teaching or industry were dismal. For lack of graduate studies or research laboratories at City College then, these possibilities barely existed. At age 19 in 1937, with a Bachelor of Science degree, and no jobs to be had in the depths of the Great Depression, I welcomed the haven that medical school would provide for four more years.

Throughout college I worked evenings, weekends, and school holidays as a salesman in men's furnishings stores. This left little time for study or sleep and none for leisure. With these earnings, a New York State Regents Scholarship of \$100 a year, no college tuition, and frugal living, I saved enough to see myself through the first half of medical school at the University of Rochester.

I enjoyed medical school and the training to become a doctor. Among my courses, biochemistry seemed rather dull. The descriptive emphasis on the constituents of tissues, blood, and urine reflected biochemistry in the United States in the 1930s. The dynamism of cellular energy exchanges and macromolecules was still unknown, and the importance of enzymes had not penetrated my course or textbook. By contrast, anatomy and physiology presented integrated and awesome structures and functions. The aberrations presented in pathology and bacteriology were absorbing, as were the responsibilities to diagnose and treat patients during the clinical years.

Did I as a medical student consider a career in research? Not really. I

expected to practice internal medicine, preferably in an academic setting; the idea of spending a significant fraction of my future days in the laboratory had no appeal. The medical school of the University of Rochester granted some students fellowships to take a year out for research. I had hoped but failed to get such an award from any of the departments. In those years, ethnic and religious barriers were formidable, even within the enlightened circle of academic science.

I did some research on my own, which grew out of curiosity about jaundice. I had noticed a slightly yellow discoloration of the whites of my eyes, and found that my blood bilirubin level was elevated and my tolerance to injected bilirubin reduced. I made similar measurements on as many medical students and patients as I could. I collected samples at odd moments and did the analyses on a borrowed bench, late at night and on weekends. The report I published (1) called attention to the frequent occurrence of high bilirubin levels and reduced capacity to eliminate bilirubin, now recognized as signs of the benign familial trait called Gilbert's Disease.

Looking back, I realized that I enjoyed collecting data. I kept on collecting bilirubin measurements during my internship year and started setting up to do more analyses in the small sickbay of a Navy ship soon after I joined it. A lucky consequence was that the publication of my student work on jaundice attracted attention and led to my transfer from sea duty to do research at the NIH, a rare assignment at that time.

## JOINING THE VITAMIN HUNTERS (1942–1945)

The Nutrition Laboratory at NIH to which I was assigned in the fall of 1942 as a commissioned officer in the U. S. Public Health Service had been started by Joseph Goldberger (1874–1929). He was among the first to recognize that a vitamin deficiency can cause an epidemic disease, and in tracking the missing vitamin in the diets of pellagra patients, he emerged as one of the greatest of the vitamin hunters. W. H. (Henry) Sebrell, whom he had trained, was now chief of the laboratory and my senior boss. The laboratory had moved in 1938 from downtown Washington to suburban Bethesda, Maryland, but some of Goldberger's animal caretakers, kitchen staff, and diet notebooks, as well as his aura were still around.

My initial project as a nutritionist was to find out why rats fed a purified ("synthetic") diet containing a sulfa drug developed a severe blood disorder in a few weeks and died. A stock animal ration or inclusion of a yeast or liver supplement in the purified diet was effective in preventing and curing the disease (2). After other vitamin hunters (3) with the use of a microbial assay had succeeded in isolating folic acid and made it available to us, we could

show that an induced deficiency of this vitamin was responsible for the sulfa drug effect.

It seemed clear that sulfa drugs, as analogues of para-aminobenzoic acid (PABA), a component of folic acid, were preventing bacteria from synthesizing this essential constituent and thus preventing their growth. We also knew that many animals rely on their intestinal bacteria for an adequate supply of folic acid and other vitamins, including vitamin K. I was therefore puzzled by a report (4) that PABA prevented the sulfa drug from producing a vitamin K deficiency even when given by injection. This result was taken to mean that the sulfa drug was not exerting its toxic effect on the intestinal bacteria, but somewhere else in the body.

I repeated the experiments with PABA injections and sulfa drugs, and developed a method to measure the amounts of vitamin K and PABA in the intestinal contents and feces. Ample quantities of vitamin K were produced by the intestinal bacteria of rats on a purified diet and this production was eliminated by sulfa drugs. As for rats injected with PABA, high levels of this substance accumulated in the intestinal contents, amounts sufficient to offset the action of the sulfa drug taken in the diet. These findings were reported in my first contribution to the *Journal of Biological Chemistry* (5), one of a very few biochemical papers from the NIH.

With the isolation of folic acid, it was apparent that virtually all the vitamins had been discovered. But we did not understand what most of the vitamins did in the body. How was folic acid serving in the growth of blood cells? What clues did the structure of folic acid offer to understanding its precise metabolic function? Could this understanding explain why sulfa drugs kill bacteria but not animal cells?

The answers to these questions, as well as to similar questions about the functions of the other vitamins, would be answered in the next two decades by enzymology. Just as the microbe hunters, who led the way in the first two decades of this century, were succeeded in the 1920s and 1930s by the vitamin hunters, so the latter would be overrun in the next two decades by the enzyme hunters.

I had come to nutrition in its twilight, decades late for the excitement and adventures of the early vitamin hunters who had solved the riddles of diseases that had plagued the world for centuries. My envy of their exploits impelled me to search for a new frontier. The discoveries of each of the vitamins—nicotinic acid, riboflavin, and thiamine in intermediary metabolism, and folic acid in nucleotide biosynthesis—became part of my heritage as I went on to learn about their biochemical functions. The rush to biochemistry depopulated the ranks of nutrition. How tragic that diet remains to this day as controversial as politics and the science of nutrition is in disarray.

## FROM RATS TO ENZYMES (1945–1947)

By 1945, with the war over, I had become bored with feeding rats variations of purified diets. I was excited reading for the first time about enzymes, coenzymes, and ATP, in papers by Otto Warburg, Otto Meyerhof, Carl Cori, Herman Kalckar, and Fritz Lipmann. I had learned nothing about these things or people in medical school. While at NIH, I was startled and fascinated by a seminar in which Edward Tatum described his and George Beadle's work with *Neurospora* mutants and their one gene-one enzyme hypothesis. I knew even less about genetics than about biochemistry.

Fortunately, I was able to persuade Dr. Sebrell to let me quit my nutritional work and go to a laboratory where I could learn about ATP and enzymes. Immediately, I apprenticed myself to Bernard Horecker, a friend at NIH, who had been studying effects of DDT on cockroaches and was returning to the subject of his doctoral dissertation, the cytochromes of cellular respiration. Bernie introduced me to succinoxidase, cytochrome c, and the Beckman Model DU spectrometer. The unsolved problem of oxidative phosphorylation seemed to me to be the most important thing to do in biochemistry.

While still in uniform, I spent the year 1946 with Severo Ochoa at New York University Medical School; it was one of the happiest and most exhilarating in my life. Never had my learning curve been so sharply exponential and sustained. And in the few waking hours outside the laboratory, Sylvy and I discovered the theater, music, and museums that are the heart-throb of New York. Despite my being a native of Brooklyn and having attended City College in uptown Manhattan, and despite Sylvy's many visits from Rochester where she grew up and studied biochemistry, we were strangers to the city.

My mission from Ochoa was to purify heart muscle aconitase. This was my first solo stab at enzyme purification. We expected to resolve the activity into two enzymes to account for the successive subtraction and readdition of a water molecule that converts citric to isocitric acid. Despite repeated failure (aconitase proved to be one enzyme), this immersion in enzymology was intoxicating. Aside from the fascination of seeing an enzyme in action, the pace of the experimental work was breathtaking. By coupling aconitase action to isocitrate dehydrogenase, spectrophotometric assays could be performed in a few minutes, and many ideas could be tested and discarded in the course of a day. Late evenings were occupied preparing a series of protocols for the following day. What a contrast with the tedious pace of nutritional experiments on rats.

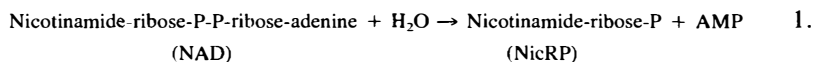
In my work on aconitase, I learned the philosophy and practice of enzyme purification. To attain the goal of a pure protein, the notebook record of an

enzyme purification should withstand the scrutiny of an auditor or bank examiner. Not that I ever regarded the enterprise as a business or banking operation. Rather, it often seemed like the ascent of an uncharted mountain: the logistics resembled supplying successively higher base camps; protein fatalities and confusing contaminants resembled the adventure of unexpected storms and hardships. Gratifying views along the way fed the anticipation of what would be seen from the top. The ultimate reward of a pure enzyme was tantamount to the unobstructed and commanding view from the summit. Beyond the grand vista and thrill of being there first, there was no need for descent, but rather the prospect of ascending even more inviting mountains, each with the promise of even grander views.

I was luckier in my second attempt at enzyme purification when I joined Ochoa and Alan Mehler, his first graduate student, in purifying the liver malic enzyme, the enzyme that converts malic to lactic acid (6). Mehler was already on the scene when I arrived in Ochoa's lab and became my indefatigable and devoted tutor. Having always been the youngest in my class, it was a shock to find that I was so far behind someone four years my junior.

To let me pursue my training and the problem of aerobic phosphorylation, the NIH extended my stay with Ochoa to a full year, and allowed me another six months in the laboratory of Carl and Gerty Cori at the Washington University Medical School in St. Louis. Right after the war, the Cori laboratory was the mecca of enzymology. There I joined a young Swedish visitor, Olov Lindberg, who was investigating a striking observation made six years earlier by Ochoa when he worked in the Cori laboratory. Liver particles metabolizing pyruvic and related acids produced inorganic pyrophosphate (PP), a compound previously unknown as a cellular constituent. We began by ruling out the possibility that PP was released from an unstable form of ATP, but then found little else to guide us.

Later, while trying to enhance the levels of respiration and coupled aerobic phosphorylation by kidney particles, we observed a strong stimulation by NAD, and discovered that the effect could be traced to AMP generated by its hydrolysis.



The AMP produced by NAD cleavage stimulated the reaction because it served as an acceptor of inorganic phosphate to form ATP. This mundane result marked the end of my search for the source of ATP in aerobic phosphorylation. The search had been doomed from the start because I was committed to finding discrete soluble enzymes that linked the synthesis of

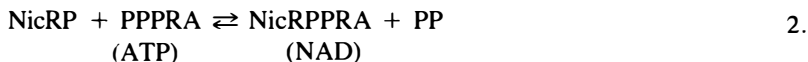
ATP to respiration. As the late Albert Lehninger recognized a few years later, these enzymes are firmly embedded in mitochondria.

## MY ROOKIE YEAR (1948)

Nineteen forty-eight, the year I set up my own biochemistry lab, was a great year for me. When I returned to the NIH, my former laboratory space in the Nutrition Division (in Building 4) was occupied. Just about then, one of the frequent organizational convulsions in the Industrial Hygiene Division (in Building 2) threatened Bernie Horecker and Leon Heppel, a close friend and medical school classmate, with a transfer to Cincinnati. Fortunately, Henry Sebrell agreed to let me start an Enzyme Section that would include the three of us in a few laboratory rooms in Building 3. (Considering the present mammoth size of the NIH, covering 300 acres and employing 13,000 people, it is hard to believe that in 1947 there were only six small buildings and that the research emphasis was still on infectious disease, dominated by a small corps of commissioned medical officers.)

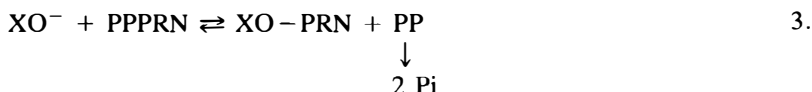
I continued the work on the rabbit kidney enzyme that Lindberg and I had discovered in St. Louis and established that it cleaves NAD at the pyrophosphate linkage (7). However, the enzyme was firmly attached to tissue particles and there was little hope of obtaining it in pure form. At the suggestion of the late Sidney Colowick and Oliver Lowry, I looked for and found a similar enzyme activity in potatoes, from which it could readily be extracted in a free, soluble form (8).

The purified enzyme cleaved not only NAD, but all nucleotides with a pyrophosphate bond. I called the enzyme *nucleotide pyrophosphatase*. By using the enzyme to cleave NADP, I could show that the position of the extra phosphate, then unknown, was part of the AMP moiety on carbon 2 of the ribose. Best of all, having isolated NicRP from NAD cleavage, I wondered whether it might serve in the synthesis of NAD. It did! Enzymes purified from yeast and liver condensed NicRP and ATP to produce not only NAD, but PP as well, the first clue to the origin of PP after years of speculation. The reaction was readily reversible and could support a vigorous exchange of PP with ATP (9).



This mechanism immediately led us to the discovery of the enzyme that synthesizes flavin adenine dinucleotide (FAD) from riboflavin phosphate and ATP (10). In the ensuing years, the mechanism of nucleotidyl transfer from a

nucleoside triphosphate for the biosynthesis of coenzymes was discovered again and again in the biosynthesis of proteins, lipids, carbohydrates, and nucleic acids. A variety of phosphoric, carboxylic, and sulfuric acids ( $\text{XO}^-$ ) accept a nucleotidyl group from a nucleoside triphosphate (PPRN) to generate an activated form of  $\text{XO}^-$  with the release of PP.



Hydrolysis of PP by a strong and ubiquitous inorganic pyrophosphatase drives these reversible condensations toward biosynthesis (11).

What a wondrous enzyme, the humble potato pyrophosphatase! It helped solve an aspect of NADP structure, set up the discovery of coenzyme biosynthesis, and with it a major theme in biochemistry, and then led me on to the enzymes that assemble DNA, genes, and chromosomes.

## OROTIC ACID IS ON THE MAIN TRACK (1953–1955)

In 1955, two years after the historic Watson and Crick reports (12) of the double helix and its implications for replication, I found an enzyme that synthesizes DNA chains from simple building blocks. Based on this chronology, it is commonly assumed that the Watson-Crick discovery spurred me to search for the enzymes of replication. But that is not the way it happened. In 1953, DNA was far from the center of my interests. The significance of the double helix did not intrude on my work until 1956, when the enzyme that assembles the nucleotide building blocks into a DNA chain was already in hand.

My interest in the replication of DNA, the focus of my research for the past 33 years, developed primarily from a fascination with enzymes. Having found an enzyme that incorporates a nucleotide into a coenzyme, I began, around 1950, to wonder about enzymes that might assemble the many nucleotides that make up the chains of nucleic acids, particularly RNA. But first we had to know the building blocks of the nucleic acids. It was not at all obvious in 1950 what they might be. Was the backbone assembled first and were the bases attached later? Was each link added to the chain as a single nucleotide? If so, was the phosphate in each component nucleotide initially attached to carbon number three or five, or to either one randomly or in a cyclic form to both?

In anticipating what the building block might be, I was influenced by what I had learned from the biosynthesis of coenzymes. I also felt that in searching for the form of the nucleotide that might serve as a building block for RNA and DNA, it would help to know how a nucleotide itself is built from simpler

molecules, and thus what its nascent form might be. Inasmuch as Jack Buchanan and Bob Greenberg were already pursuing purine biosynthesis, I decided to go after the pyrimidines.

During a brief interlude, I acted on the hunch that biosynthesis of the phosphodiester bond, accessible in phospholipids, might offer a model for building the backbone of nucleic acids. In exploratory experiments with [ $^{32}\text{P}$ ]- $\alpha$ -glycerophosphate and [ $^{14}\text{C}$ ]-phosphoryl choline, I could find no evidence for their condensation to form the diester (glycerophosphoryl choline), but I did stumble on the formation of phosphatidic acid and phosphatidyl choline in the cell-free extract (13). I worked out the enzymatic synthesis of phosphatidic acid (14), the key precursor of phospholipids, but still was eager to get away from greasy molecules and return to pyrimidines and the aqueous phase. In the future, I would not rely on intuition about model systems, but would head toward an objective directly.

Osamu Hayaishi came as a postdoctoral fellow in 1950 experienced in the use of soil bacterial enrichment cultures. Among the huge variety of species in soil, at least one can be found that will respond to virtually every natural organic compound and use it as a source of carbon and energy. Believing too that reversibility of metabolic pathways might provide clues to biosynthesis, we examined the breakdown of uracil and thymine in extracts of bacteria isolated from soil by aerobic enrichment on these pyrimidines. Uracil and thymine were converted to the corresponding barbiturates, not at all promising as biosynthetic precursors (15). But the next year, during my first visit to California, H. A. Barker helped me find an anaerobe in San Francisco Bay mud that consumed orotic acid. Back at NIH, with the participation of Irving Lieberman, who had been a student of Barker, studies of this organism identified as metabolic products dihydroorotic acid and carbamyl aspartate (16), which later proved to be intermediates in the biosynthesis of orotic acid.

Orotic acid was known from intact cell studies to be a precursor of nucleic acid pyrimidines, but it was uncertain whether it was on the main track or connected to it by a spur. With orotic acid tagged in its carboxyl group, the release of  $\text{CO}_2$  to form uracil might lead us to the enzyme that took orotic "up" to nucleic acid.  $\text{CO}_2$  release by extracts from yeast or liver was terribly feeble, yet showed a tantalizing requirement for ATP and ribose 5P. One happy day, instead of using extracts of either yeast or liver, I combined them. The reaction was explosive, hundreds of times greater than before, one of those rare moments in a scientific lifetime.

The enzyme abundant in liver extracts transferred a PP group from ATP to carbon 1 of ribose 5P to produce the novel phosphoribosyl pyrophosphate (PRPP) (17), later recognized as the key precursor of purine nucleotides, histidine, tryptophan, and NAD. The enzyme in yeast extracts (actually two

enzymes) formed orotidine 5P, which then was decarboxylated to UMP (18), the direct precursor of all the nucleic acid pyrimidines (Figure 1).

The transfer of pyrophosphate to ribose 5P entails an attack on the middle phosphate of ATP, as Gobind Khorana showed during one of his whirlwind and productive visits to my lab (19). (Other examples of this unusual reaction are the synthesis of thiamine PP, and guanosine tetraphosphate.) PRPP synthetase remains one of my favorite enzymes. As I wrote in the 1975 Festschrift for Ochoa (*Reflections on Biochemistry*, Pergamon Press): "Most of us anticipated that ribosyl activation for nucleotide biosynthesis would use the same device of phosphorylation, so well known for glucose. But the novelty of pyrophosphorylation used by this enzyme (coupled with elimination of inorganic pyrophosphate upon subsequent condensations) established my unalloyed awe for the ingenuity and fitness of an enzyme."

Knowing that PRPP enables a free pyrimidine (orotic acid) to be converted directly to a nucleotide, we sought and found enzymes that used PRPP to convert free purines (adenine, hypoxanthine, guanine) directly to nucleotides (20). Yet, I also knew from Buchanan's and Greenberg's studies (21, 22) that a purine ring is assembled from the very outset attached to ribose phosphate (later shown to be derived from PRPP). These facts, coupled with the knowledge that nucleotides can be formed from nucleosides by kinases, made it clear to me that cells have alternate pathways to the biosynthesis of nucleotides: salvage of preformed bases and nucleosides, and de novo routes from smaller molecules (e.g. sugar phosphates, amino acids, ammonia, one-carbon units). We have since realized that the role of salvage pathways can be as vital as the de novo pathways even under normal conditions when the de novo routes are not blocked by mutation, drugs, disease, or excessive traffic (23).

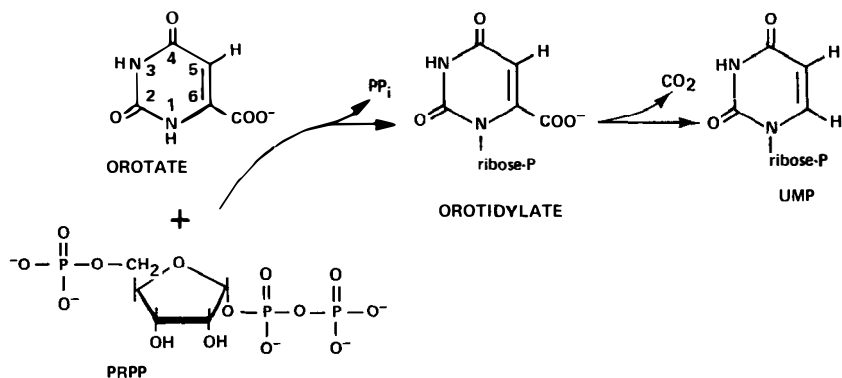


Figure 1 Condensation of orotic acid with PRPP produces the nucleotide, orotidylate (orotidine 5P), which upon decarboxylation generates uridine 5P (UMP).

## DISCOVERY OF DNA POLYMERASE (1955–1959)

Having learned how the likely nucleotide building blocks of nucleic acids are synthesized and activated in cells, it seemed natural that in 1954 I would look for the enzymes that assemble them into RNA and DNA. Such an attempt might have been considered by some as audacious. Synthesis of starch and fat, once regarded as impossible outside the living cell, had been achieved with enzymes in the test tube. But, the monotonous array of sugar units in starch or the acetic acid units in fat was a far cry from the assembly of DNA, thousands of times larger and genetically precise.

Yet, I was only following the classical biochemical traditions practiced by my teachers. It always seemed to me that a biochemist devoted to enzymes could, if persistent, reconstitute any metabolic event in the test tube as well as the cell does it. In fact better! Without the constraints under which an intact cell must operate, the biochemist can manipulate the concentrations of substrates and enzymes and arrange the medium around them to favor the reaction of his choice.

I have adhered to the rule that all chemical reactions in the cell proceed through the catalysis and control of enzymes. Once, in a seminar on the enzymes that degrade orotic acid (16), I realized that my audience in the Washington University chemistry department was drifting away. In a last-ditch attempt to gain their attention, I pronounced loudly that every chemical event in the cell depends on the action of an enzyme. At that point, Joseph Kennedy, the brilliant young chairman, awoke: "Do you mean to tell us that something as simple as the hydration of carbon dioxide (to form bicarbonate) needs an enzyme?" The Lord had delivered him into my hands. "Yes, Joe, cells have an enzyme, called carbonic anhydrase. It enhances the rate of that reaction more than a million-fold."

By 1954, the rapidly growing *Escherichia coli* cell had become a favored object of biochemical and genetic studies, and for me had replaced yeast and animal tissues as the preferred source of enzymes. To explore the synthesis of RNA, Uri Littauer, a postdoctoral fellow, and I prepared [ $^{14}\text{C}$ -adenine]-ATP and maintained it as ATP with a regenerating system. Upon incubation with an *E. coli* extract, a small but significant amount of the radioactivity was incorporated into an acid-insoluble form, presumably RNA, and we proceeded eagerly to purify the activity responsible.

I also pursued the synthesis of DNA. Here, I had the invaluable help of Morris Friedkin, who had synthesized  $^{14}\text{C}$ -thymidine and was studying its uptake into the DNA of rabbit bone marrow or onion root tip cells. Disinclined to work with cell-free extracts, he generously saved the spent reaction fluid from which I recovered radioactive thymidine to use in trials with extracts of *E. coli*.

The results were mixed. Very little thymidine was incorporated into the acid-insoluble form indicative of DNA, only about 50 cpm out of the million with which we started. On the other hand, 5–10% of the thymidine was converted to novel soluble forms that resembled the phosphorylated states of the nucleotide building blocks, possibly better precursors than thymidine for DNA synthesis.

At this juncture, Herman Kalckar on a visit to St. Louis brought us the startling and unsettling news that Ochoa and Marianne Grunberg-Manago, a postdoctoral fellow, had just discovered the enzymatic synthesis of RNA. It was for them a totally unexpected finding made while exploring aerobic phosphorylation in extracts of *Azotobacter vinelandii*. They observed an exchange of phosphate into ADP and the reversible conversion of ADP (or other nucleoside diphosphates) into RNA-like chains (24) and they named the enzyme polynucleotide phosphorylase.

On the strength of this new information, we shifted to using ADP rather than ATP in our studies with *E. coli*. The rate and extent of reaction were far greater and we readily purified the enzyme involved (25). We had made a classic blunder. Accounting for a phenomenon does not insure that it is the only or the best explanation of it. In this instance, we were diverted from the discovery of RNA polymerase, which depends on ATP. By switching to ADP, we tracked the synthetic activity of polynucleotide phosphorylase and missed the key enzyme for gene transcription.

Ten months passed before I repeated the experiment of converting radioactive thymidine to an acid-insoluble form. Once again, only a tiny amount of this presumed precursor was converted. But several things were different. For one, the radioactivity of the thymidine happened to be three times as great and so the results seemed more impressive. For another, believing I had lost out on the synthesis of RNA, the synthesis of DNA became a more precious goal. Finally, I exposed the product this time to pancreatic DNase and found that it became acid-soluble, a strong indication that it was DNA.

Even before I calculated the DNase results, I stopped to tell Bob Lehman about them. Although his postdoctoral problem was well started, he was eager to switch to DNA synthesis. Progress was rapid. Bob soon found that thymidine phosphate was a far better precursor than thymidine and later showed that thymidine triphosphate was much better still. With improvements in the assay of DNA synthesis by these crude extracts, our goal was to purify the enzyme that assembled nucleotides into a DNA chain, the enzyme we would name DNA polymerase (26, 27).

The most complex and revealing insights into the reaction would come from exploring the function of the DNA that I had included in the reaction mixture in my earliest attempt to incorporate thymidine into DNA. Some assume that DNA was included to serve as a template and that its primer role emerged many years later. Not so. I added DNA expecting that it would serve

as a primer for growth of a DNA chain, because I was influenced by the Cori work on the growth of carbohydrate chains by glycogen phosphorylase. I never thought that I would discover a phenomenon utterly unprecedented in biochemistry: an absolute dependence of an enzyme for instruction by its substrate serving as a template.

I had added DNA for another reason. Nuclease action in the extracts was rampant, and I wanted a pool of DNA to surround the newly incorporated thymidine and protect at least some of it. Only later did Lehman and I learn with elation that the added DNA fulfilled two other essential roles. It indeed served as a template and also as a source of the missing nucleotides. The DNA was cleaved by DNases in the extract to nucleotides. These were converted by ATP and five kinases in the extract to the di- and triphosphates of the A, G, C, and T deoxyribonucleotides, which were then still unknown.

Maurice Bessman, Steve Zimmerman, and Julius Adler joined Bob Lehman, Sylvvy, Ernie Simms (my research assistant), and me and occasionally one or two others, all in a small laboratory, only about 20 by 20 feet. Crowded and excited, we shared ideas, reagents, and data. The sum of our efforts was far greater than if we had been diluted into a larger room or separated by walls.

## CREATION OF LIFE IN THE TEST TUBE (1960–1967)

With purified DNA polymerase, we could show that the DNA product reflected the base composition of the template and the frequencies of the 16 possible dinucleotides. The “nearest-neighbor” sequence method, which we devised to determine the dinucleotide frequencies, also revealed that the two strands of the double helix have opposite polarities, a structural feature that had not been experimentally demonstrated up to that time (28).

We also made the unexpected discovery that the enzyme, in the apparent absence of any template would, after a considerable delay, make DNA-like polymers of simple composition (29, 30): the alternating copolymers polydA•dT and polydG•dC and the homopolymer pairs of polydA with polydT and of polydG with polydC. These polymers, once made, proved to be superior templates and have been widely used in DNA chemistry and biology. Generation of the polymers *de novo* could be ascribed to the reiterative replication of short sequences in the immeasurably small amounts of DNA that contaminate a polymerase preparation (31, 32).

For more than 10 years, I had to find excuses at the end of every seminar to explain why the DNA product had no biologic activity. If the template had been copied accurately, why were we unsuccessful in all our attempts to multiply the transforming factor activity of DNA from *Pneumococcus*, *Hemophilus*, and *Bacillus* species? Finally, with the arrival of ligase in 1967, a crucial test could be made. [The enzyme had been discovered that year in

five laboratories: those of Martin Gellert, Charles Richardson, and Jerard Hurwitz, in Lehman's next door, and in mine by Nicholas Cozzarelli.] Mehran Goulian and I could replicate the single-stranded circle of phage  $\phi$ X174 with DNA polymerase and then seal the complementary product with ligase. The circular product was isolated and then replicated to produce a circular copy of the original viral strand, which could be assayed for infectivity in *E. coli* (33). We found the completely synthetic viral strand to be as infectious as that of the phage DNA with which we started!

After so many years of trying, we had finally done it. We had gotten DNA polymerase to assemble a 5000-nucleotide DNA chain with the identical form, composition, and genetic activity of DNA from a natural virus. All the enzyme needed was the four common building blocks: A, G, T, and C. At that moment, it seemed there were no major impediments to the synthesis of DNA, genes, and chromosomes. The way was open to create novel DNA and genes by manipulating the building blocks and their templates.

In a very small way, we were observers of something akin to what those at Alamogordo on a July day in 1945 witnessed in the explosive force of the atomic nucleus. Harnessing the enzymic powers of the cellular nucleus had neither the dramatic staging of light and sound nor the stunningly apparent global consequences. Yet, this demonstration of our power with enzymes that build and link DNA chains would soon help others forge a different revolution, the engineering of genes and modification of species.

A hundred newspaper and television reporters and photographers came to a press conference called by the Stanford News Bureau on December 14, 1967, because of many inquiries about the paper we had just published in that month's issue of the *Proceedings of the National Academy of Sciences* (34). The title was: "Enzymatic Synthesis of DNA, XXIV. Synthesis of Infectious Phage  $\phi$ X174 DNA." To the editors who sent the newsmen, it seemed that a virus had been synthesized and life created in the test tube.

At the news conference, I tried to explain why the definition of life and a living molecule is so elusive. Afterwards, I overheard a reporter on the telephone to his office: "It's not what we expected. They haven't made a virus. It's only a molecule, a short chain of DNA. They've been making DNA in the test tube for 12 years." Hairy little monsters had not been created in the test tube. Yet, the story rated banner headlines worldwide and a newspaper article on January 4, 1968, was titled: "Creation of Life Rates Best of Science Stories in 1967." In smaller type: "Human Heart Transplant Second."

## PROOFREADING AND EDITING BY A REPLICATING ENZYME (1967–1971)

Knowing that DNA polymerase synthesized a chain in the 5' to 3' direction, it made no sense to me then that the enzyme degraded the very 3'-end of the

chain it would normally be extending. In the absence of the nucleotide building blocks needed for synthesis, nucleotide units were cleaved slowly and serially from the 3' end of a DNA chain. Then a simple fact about the nuclease gave us our best clue. Douglas Brutlag observed that the degrading activity was far more potent on a single strand of DNA than on the usual double-stranded form. This preference became extreme when the temperature of the reaction was lowered, presumably because the ends of duplex DNA are less frayed at lower temperatures.

Why should a loose primer end be a substrate for degradation by a synthesizing enzyme? We prepared a variety of duplex DNAs in which a few residues at the primer end of a chain were not matched to the other strand. The mismatched residues were removed immediately, after which the others were removed far more slowly. When deoxynucleoside triphosphates were supplied to permit chain extension, the mismatched residues were still removed quickly, but now the subjacent nucleotides remained intact and were extended by synthesis (35).

Thus, the enzyme removes all mismatched units, permitting fresh units to be added to the growing chain end only when it is correctly matched to the template chain. We could infer that if the synthesizing enzyme were to make a rare mistake during elongation of a chain, such as inserting a C opposite an A (estimated to happen once in 10,000 times), it would remove the mismatched C before proceeding with extension of the chain. This astonishing proofreading ability of the enzyme, coupled with its fine discrimination in the initial choice of correct building blocks during synthesis, reduces errors in the overall process of replication to one in 10 million.

Having finally made sense of why an activity that degrades DNA is part of the very enzyme that makes it, we were unprepared for the paradoxical observation by Lehman (36) that the nuclease action of DNA polymerase on double-stranded DNA was enhanced tenfold when all four building blocks required for synthesis (i.e. A, T, G, and C) were present. How could synthesis be enhancing degradation? After all, we had observed earlier that synthesis extends the primer end of a chain and thereby protects it from nuclease action.

The solution came from Edward Reich and his colleagues (37) and from Murray Deutscher in my laboratory (38), showing that nuclease activity in DNA polymerase persists even with the 3'-end blocked by an analogue or phosphate. A separate domain in the enzyme removes nucleotides from the 5'-end of a chain.

Now we could explain how the four building blocks, and the synthesis they make possible, enhance nuclease action by DNA polymerase (39). By removal of DNA from the 5'-end at a nick, a stretch of template becomes exposed for pairing with a substrate nucleotide and further synthesis. In its synthetic progress along the template, the polymerase is brought up to a 5'-end of the

chain, which it then degrades. It was immediately obvious how this “nick translation” by polymerase could be useful in the repair of lesions in DNA (40), and as we recognized some years later, could perform an essential step in replication by removing the RNA that initiates the start of a DNA chain.

## DNA POLYMERASE UNDER INDICTMENT (1970–1972)

DNA polymerase was called a “red herring” and charged by *Nature New Biology* in a series of editorials with masquerading as a replication enzyme (41). The replicative role of DNA polymerase was questioned because of the Cairns mutant of *E. coli* (42), which appeared to lack the enzyme and yet grew and multiplied at a normal rate. In addition to the apparent dispensability of DNA polymerase for cell multiplication and its more estimable qualifications as a repair enzyme, genes were being discovered (designated *dnaA*, *dnaB*, *dnaC*, etc) that strongly implicated many other proteins as essential for a replication process far more complex than had been imagined.

The rising skepticism about the importance of DNA polymerase was fanned by the *Nature New Biology* vendetta. Not only was the enzyme attacked, but the basic mechanism, the building blocks, and the assays of DNA synthesis were judged to have prevented the discovery of the true DNA-replicating enzymes. At this juncture, my middle son, Tom, entered the fray. (I was at the Molecular Biology Laboratory in Cambridge, England, in the second half of a sabbatical year devoted to membranes.) An injury to his hand prevented him from continuing his career as a cellist at the Juilliard School, and he was disturbed by disparaging comments about DNA polymerase in his biology course at Columbia College where he was also a full-time student. Despite lack of laboratory experience, he found, within a few weeks, a DNA polymerase in *E. coli* cells, distinct from the one I had discovered. The new activity, named DNA polymerase II (pol II) (43), was clearly different from the “classic” DNA polymerase (pol I) and from still another, DNA polymerase III (pol III) (44), which he discovered in the course of purifying pol II. Subsequently, he and Malcolm Gefter located the gene for pol III and showed that conditionally lethal mutations in this gene blocked DNA replication (45). Pol III, in a far more elaborate form, was to gain recognition as the central enzyme of DNA replication in *E. coli*.

All three polymerases, although differing significantly in structure, proved to be virtually identical in their mechanisms of DNA synthesis, proofreading, and use of the same building blocks. The maligned polymerase (pol I) became the prototype for all DNA polymerases in plants, animals, and viruses, as well as in *E. coli*. The gloomy prophecies of *Nature New Biology* soon disappeared, as did the magazine itself.

## HOW DNA CHAINS ARE STARTED (1971–1975)

Despite the excitement over the synthesis of a chain of infectious viral DNA, I had felt a certain uneasiness. One of the inferences drawn from the replication of a single-stranded, circular template was that DNA polymerase I could start a new chain. Yet we were never able to find direct proof of this. Moreover, we had observed that replication of the circular template was far more efficient if a small amount of boiled *E. coli* extract was present. Although it seemed unlikely that a random fragment of DNA in the extract would match the viral DNA template accurately enough to serve as a primer, this possibility became a reality. DNA polymerase removed the unmatched regions of the fragment by proofreading at the 3' end; with generous editing at the 5' end, no trace of the fragment remained in the synthetic product.

We were left with the question of how DNA chains are started, how a single-stranded, circular viral DNA is converted to the duplex form upon entering the cell, how nascent chains are initiated in the replication of virtually all chromosomes. Indeed, Reiji Okazaki had shown earlier (46) that chains are started not just once, at the beginning of the chromosome, but repeatedly in staccato fashion during the progress of replication.

After several years of unproductive attempts, I recognized a basic flaw in our work, how hopeless it was to answer the question about chain starts with the DNA we were using as template and primer. A tenet I was taught and to which I had faithfully adhered is that one must purify an enzyme to understand what it does. An aphorism attributed to me, but actually due to Efraim Racker, is: "Don't waste clean thinking on dirty enzymes." Another basic tenet of enzymology, too obvious, it would seem, to mention, is that one must provide the enzyme, clean or not, with a pure substrate.

The blunder we had made for too many years was accepting the DNA extracted from bacterial and animal cells as an adequate substrate for the enzymes of replication. The huge chromosomes are very fragile and the mechanical forces of flow and mixing used during isolation are violent enough to reduce the DNA to a heterogeneous collection of damaged fragments. In short, we were giving our relatively clean enzyme a very "dirty" substrate.

When I finally recognized the futility of searching for the replication enzymes with bacterial DNA, let alone animal DNA, I also realized that we had been ignoring a proper DNA substrate, the chromosome of a tiny bacteriophage. I recalled belatedly the virtues of the intact, clean, phage chromosome that four years earlier had served us in demonstrating the synthesis of infectious DNA by DNA polymerase I. As small, single-stranded circles, we could actually view them with an electron microscope and verify that in a purified sample, they were intact, homogeneous, and uncontaminated by

fragments of the bacterial host DNA. We also knew that immediately upon entering the cell, the phage DNA is converted by bacterial enzymes to a double-stranded circle, an event we could easily assess. Probing how a new phage circle is started and completed might illuminate the intricate enzymatic machinery the cell uses to replicate its own chromosome.

We could use the chromosome of either of the two classes of small phages, the icosahedral  $\phi$ X174 or the filamentous M13. Luckily, as events later proved, I chose to work on M13 and within a week or two switched the efforts of my entire group to the various stages of its life cycle. I have sometimes felt wistful reflecting on the boldness of that move and the exciting events in the weeks that followed.

Being preoccupied with the initial event in M13 replication enabled me to connect three otherwise unrelated facts and arrive at an idea as to how a DNA chain might get started. For one, RNA polymerase, unlike DNA polymerase, can start chains. Furthermore, DNA polymerase, while routinely excluding ribonucleotides in assembling a DNA chain, does accept an RNA chain end matched to a DNA template as a primer for extension in DNA synthesis. Finally, DNA polymerase I has an editing function, which can remove something foreign, like thymine dimers and RNA from the start of a DNA chain, and replace it with proper DNA.

Might RNA polymerase make a short piece of RNA on single-stranded M13, which DNA polymerase could use to start a DNA chain? Then, when the enzyme had come full circle in copying the available template, its editing system would erase the RNA and synthesize DNA in its place. We could test this hypothesis by using rifampicin to inhibit RNA polymerase *in vivo* (47). When Doug Brutlag did so, the M13 circle was not replicated! We went on to show with cell extracts and partially purified enzymes that RNA polymerase initiates DNA replication by forming a primer RNA for covalent attachment of the deoxyribonucleotide that starts the new DNA chain (48).

There was one discordant note. Rifampicin did not prevent the conversion of the phage  $\phi$ X174 single-stranded circle to its duplex form, nor did it interrupt the ongoing replication of the *E. coli* chromosome, despite the repeated initiations of DNA strands presumed to be occurring at the growing fork. Either RNA priming was of limited significance, or another mode of RNA synthesis, independent of RNA polymerase, existed. As we probed the initiation on  $\phi$ X174 circles, it became clear that this virus, instead of relying on RNA polymerase, exploits the extraordinarily complex assembly of initiation proteins that the cell uses for replicating its own chromosome.

## PRIMOSOMES, HELICASES, AND REPLISOMES (1972–)

After a 10-year drought of discoveries of new enzymes, they now came in a torrent, and in 1972 a bright and boistrous group, led by Randy Schekman and

Bill Wickner, was there to collect and sort them. Upon fractionating the components responsible for conversion of the single-stranded circle of  $\phi$ X174 to the duplex form, we could separate them into two groups, one that primed the start of a chain and the other that extended it. Then, as we tried to purify each of these fractions, they splintered into many separate components. The joy of uncovering the trails to so many novel proteins soon gave way to the discouragement of being unable to track down any one of them. We judged there might be as many as eight different proteins, all scarce, that were needed to make the tiny bit of RNA that primed the synthesis of a DNA chain; the DNA polymerase seemed just as complex. What were all these proteins and what was each of them doing?

A major assist came from New Haven sewage via Nigel Godson, who discovered in it a new phage (G4) that resembled  $\phi$ X174 (49). Replication of G4 DNA required only only three of the eight fractions needed by  $\phi$ X174 (50). One of the fractions was purified easily because it withstood heating. It was the single-strand binding protein (SSB) (51), which coats the DNA, except at a region that forms a duplex, hairpinlike structure and is used by the second fraction as a template to synthesize a stretch of primer RNA. This protein, known to complement the deficiency of cell extracts of *dnaG* mutants, was named primase (52). The third fraction was DNA polymerase III in a complex, unstable form (53) with many auxiliary units that clamp the polymerase to the template and enable it to replicate great lengths of DNA with astonishing speed and accuracy. Replication of G4 DNA thus provided us with our best assays for purifying each of these components: SSB, primase, and the super polymerase we now called DNA polymerase III holoenzyme.

We could now return to  $\phi$ X174 and begin to explain the molecular operations of the multiprotein assembly (called a primosome) (54) that starts a DNA chain. The image of a locomotive seemed helpful for a time in accounting for primosome actions. The engine, protein  $\eta'$  (55) (its gene unknown to this day), powered by ATP energy, is a helicase (56, 57); it unzippers the DNA duplex and is equipped with a cowcatcher to remove SSB in its path. Another protein, *dnaB*, is both helicase (58, 59) and engineer (60), using ATP to locate or shape a section of DNA track upon which primase will find it possible to lay down a short stretch of RNA, which will then attract DNA polymerase to start a DNA chain. The primosome is translocated on DNA in only one direction, the one that keeps it at the advancing fork of a replicating chromosome (61).

Another awesome property of the primosome is the persistence of its attachment to the  $\phi$ X174 DNA circle even after the duplex circle has been completed (62). The attached primosome directs the next replication event and is used over and over again as part of a stamping machine to generate the many duplex forms needed for transcription into messages for the 10 proteins encoded by the phage DNA.

To multiply duplex circles (Figure 2), the phage employs a single gene (gene A) of its own and relies on host proteins to do the rest. The isolated gene A protein breaks the viral strand backbone at a particular diester bond and becomes covalently attached to the 5'-end of the break (63). In so doing, it makes the 3'-end available as a primer for DNA polymerase III holoenzyme replication. For lack of exposed template at this nick, the phage exploits the host rep protein, a helicase, to unzip the duplex (64, 65). This was the first occasion in which a helicase was seen in its role of opening of a duplex for an advancing replication fork. Coordinate helicase and polymerase actions generate single-stranded viral circles repeatedly by a mechanism called rolling-circle replication (66). Each completed circle is then replicated to form a duplex in the same way as the one originally injected by the phage.

Based on these insights gained from the proteins involved in the replication of the small phages, we now propose that progress of replication of the host chromosome (Figure 3) (67) depends on helicases, SSB, and topoisomerases to prepare the templates for the continuous synthesis of a leading strand and the discontinuous synthesis of the other. We regard the polymerase III holoenzyme as a rather loose assembly of many auxiliary units attached

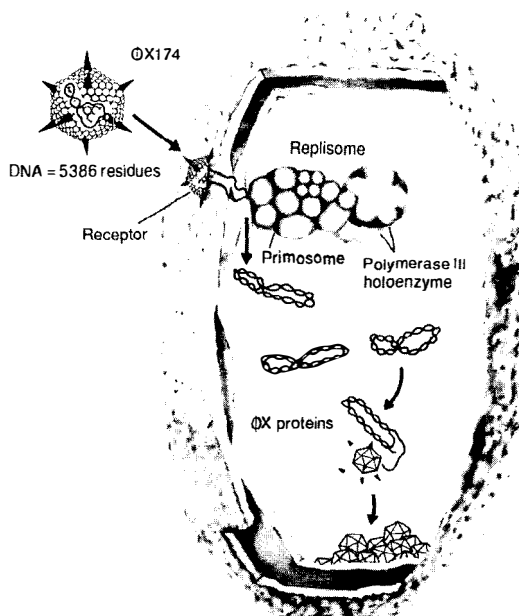


Figure 2 Life cycle of phage  $\phi$ X174 in *E. coli*. Conversion of the viral circle to a duplex is followed by multiplication of the duplexes by a rolling-circle mechanism.

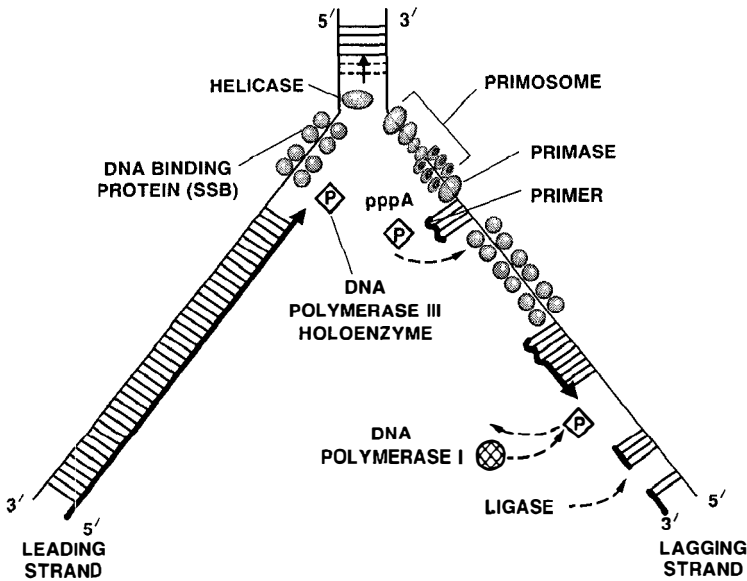
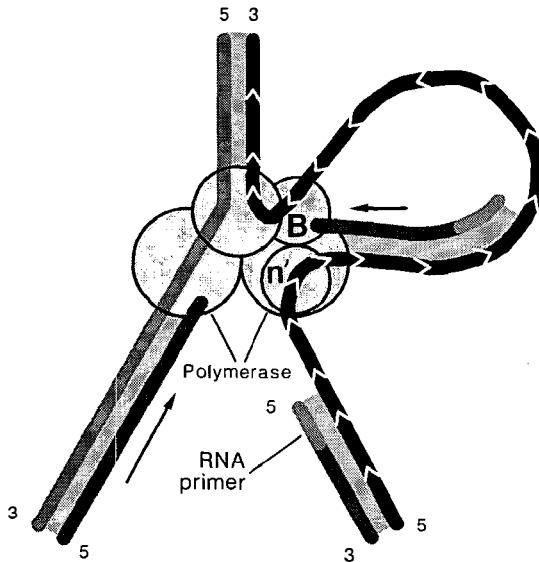


Figure 3 Scheme for enzymes operating at a replication fork in *E. coli*: continuous synthesis of a leading strand and RNA-primed discontinuous synthesis of the other strand.

asymmetrically to a pair of polymerase cores. One arm appears suited to the highly processive synthesis of the leading strand and the other arm to the discontinuous synthesis of the other strand (68, 69).

We now wonder whether essentially concurrent replication of both strands might be achieved were priming of nascent fragments of discontinuous synthesis integrated with continuous strand synthesis. A replisome comprising the holoenzyme, primosome, and helicases might periodically generate a loop in the lagging strand template to place it in the same orientation as the leading strand at the fork (Figure 4) (67).

We had been aware that the primosome and the primase in it moved in opposite directions on a DNA chain. With additional knowledge, the difficulty grew. The isolated *dnaB* component was found to move in the direction of the primosome (58), whereas the *n'* component by itself moved in the opposite (chain elongation) direction (57). A smoldering annoyance had become a serious paradox. How could integral parts of the primosome locomotive move in opposite directions on a fixed track of DNA? The analogy would have to be scrapped. Instead, it now makes more sense to invert the relative movements of the primosome and DNA and regard the primosome machine as fixed in place with the DNA chains being drawn through it (Figure 4) (57). This view also offers an attractive mechanism for coordinating the



**Figure 4** Hypothetical view of the replisome as a fixed machine through which a loop of DNA is pulled to provide for essentially concurrent replication of both strands. As the DNA is pulled through the primosome, the *n'* protein is translocated on the DNA in the direction of chain elongation and the B protein in the opposite direction.

helicase, priming, and replication actions at the advancing fork of a chromosome.

## INITIATION OF CHROMOSOMES (1979–)

An aspect of replication that has long intrigued me is how an increase in *E. coli* cell mass triggers the initiation of replication that commits the cell to start a new cycle. What is the biochemistry of the replication switch, which in *E. coli* regulates the cell cycle and in eukaryotes responds to signals that turn the embryonic cell to a quiescent adult, or the quiescent cell to proliferation? With the cloning of the highly conserved, unique, 245–base pair chromosomal origin (*oriC*) in plasmids, we were afforded a substrate with which to seek the comparably complex, conserved multiprotein system that uses *oriC* to initiate a cycle of replication. After 10 man-years of fruitless effort to obtain a cell-free initiation system, Bob Fuller and Jon Kaguni were the ones who finally succeeded (70). Two apparently illogical maneuvers were essential. One was the inclusion of a hydrophilic polymer (e.g. polyethylene glycol) at

high levels which, as we later realized, acted by a “macromolecular crowding” effect, which concentrates the numerous proteins and DNA into a small volume. The other was subjecting an inert lysate to a refined ammonium sulfate fractionation, a trick that had worked for me 30 years earlier in the discovery of the yeast enzyme that converts NAD to NADP (71). In the active fraction the numerous required proteins are concentrated and potent inhibitors are excluded.

The proteins that we found responsible for initiating replication at *oriC* include *initiation proteins* (particularly *dnaA* protein) that recognize supercoiled *oriC*, alter its structural conformation, and lead to its further opening by *dnaB* helicase action, *specificity proteins* that suppress potential origins elsewhere on the chromosome, and the *replication proteins* that prime and elongate chains on the opened plasmid and propel two forks in opposite directions (72, 73).

The motif we are finding in the mechanism for initiation of the *E. coli* chromosome (Figure 5) seems to apply to a wide variety of bacteria and their plasmids and phages (74). Control of *dnaA* protein by ATP-ADP cycling (75) and by binding to the acidic headgroups of a fluid membrane (76), and activation of an inert origin in an overly relaxed supercoil by nearby transcription (77) are among the factors already discovered that influence initiation, and likely more will be found as we explore this crucial event in the cell cycle.

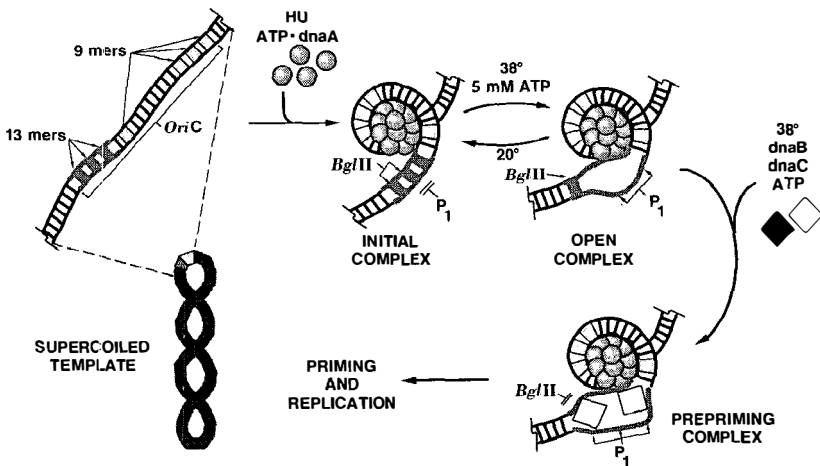


Figure 5 Scheme for early events in initiation of replication at the origin of the *E. coli* chromosome operating in a plasmid.

## SPORULATION AND GERMINATION (1962–1970)

Those familiar with my research are aware of my intense concentration on a single subject—the enzymatic synthesis of DNA—and the blinders I have worn to maintain this focus. Nearly forgotten now by us all are the eight years, in the midst of the DNA work, when half of my research effort was devoted to an arcane subject, the development of spores (78).

During my tenure as chairman of a department of microbiology (1953–1959), I had become interested again in spores as agents of disease: anthrax, tetanus, botulism. I constantly remembered with deep anguish the ghastly *Clostridium perfringens* (gas gangrene) spore that killed my mother within a day of a “routine” gall bladder operation in 1939. Now I could look beyond the “bad” spores to the vast array of innocent species whose mysterious biology and biochemistry fascinated me.

How is a spore made and how does its chemical organization endow it with astonishing abilities: dormancy and resistance to extremes of heat, desiccation, disinfectants, and ultraviolet rays lethal to the cell? How does a spore, after years of hibernation, respond instantly to a substance that signals conditions are right for growth into a new cell? I believed then, and still do, that this knowledge would contribute in a major way toward understanding the embryonic development of animals and their response to environmental stresses.

During the eight-year period in which a succession of students and postdoctoral fellows (including Pieter Bosen, Pierre Chambon, Murray Deutscher, Arturo Falaschi, David Nelson, Tuneko Okazaki, Peter Setlow, Jim Spudich, Henrique Tono, and Jim Vary) worked on spores, we published 26 papers and still were making little progress toward answering the global questions that had attracted me.

I abandoned the spore work when I came to realize how much more complex the problems were than I had imagined and that hardly anyone else in the world seemed to care. The little research on spores, then and even now, was largely of a practical nature: how to destroy spores in food canning or how to use them as pesticides on crops.

Beyond the discouragement and loneliness of working on a tough problem in an unfashionable area was the distraction of having the other half of my research group of eight or so engaged in the more glamorous and productive work on DNA replication. Because of my own ambivalence, I offered no resistance on occasions when a member of the sporulation group defected to the replication team. Finally, after this eight-year siege, I too gave up. Eventually only Peter Setlow has continued a biochemical interest in spores.

Progress in science depends on how vigorously the field is cultivated. In contrast with sporulation, interest in cancer is enormous. Hundreds of labora-

tories worldwide attract many thousands of scientists, including the brightest, to unravel the processes responsible for malignant growth. Yet studies of sporulation are also deserving of resources and talents. Sporulation, dormancy, and germination are fundamental processes in nature, more accessible to incisive examination, and, if better understood, might yield as much information relevant to the cancer process as some of the massive programs on tumor-bearing animals.

## SCIENTIST, TEACHER, AUTHOR, CHAIRMAN: IN WHAT ORDER?

In May 1988, when my former and present students and colleagues gathered in San Francisco for a gala 70th birthday party, I thought it would be fun to select from the 30 or more enzymes I had worked with, the 10 that I favored most. I was surprised to find that 6 of the 10 were discovered in the brief period from 1948 to 1955: nucleotide pyrophosphatase (8), NAD synthetase (9), phosphatidic acid synthetase (14), PRPP synthetase (17), polyphosphate synthetase (79), and DNA polymerase (26). That left only 4 to be selected from more than 20 enzymes that appeared in the next 30+ years. Inasmuch as some of the enzymes omitted from the top 10 are far more deserving of selection than some of the chosen ones, it is clear that the basis for the choice of the first 6 was largely sentimental. I was most attached to those enzymes that came during the time of my life when I collected the data myself, from conception to delivery.

In my marriage to enzymes, I have found a level of complexity that suits me. I feel ill at ease grappling with the operations of a cell, let alone those of a multicellular creature. I also feel inadequate in probing the fine chemistry of small molecules. Becoming familiar with the personality of an enzyme performing in a major synthetic pathway is just right. To gain this intimacy, the enzyme must first be purified and I have never felt unrewarded for any effort expended this way.

I once shocked the Dean of the Washington University School of Medicine by telling him that my prime interest as Chairman of the Department of Microbiology was to do and foster research rather than teach. It has never been otherwise. Experiments are far more consuming and fulfilling for me than any form of teaching. Still, I have enjoyed a rather modest amount of formal lecture and laboratory instruction and have done it conscientiously. For the student, didactic teaching fails without the infusion of scientific skepticism and a fervor for new knowledge, and these things are naturally conveyed by someone dedicated to research. For me, some 10 lectures a year freshen my awareness of basic subjects, and on one occasion the preparation

of a laboratory exercise on DNA opened a major avenue for my experimental work.

The most rewarding teaching for me has been in the intimate, daily contact with graduate and postdoctoral students. Well over a hundred of them spent from two to five years in my laboratory and were exposed to my tastes and my obsession with the use of time. I felt closest to those who shared my devotion to enzymes and my concern with the productive use of our most precious resource: each of the hours and days that so quickly stretch into the few years of a creative life. I recall in 1948 relating to Sidney Colowick and Ollie Lowry (both senior to me in age and experience) my failure in purifying an enzyme by a certain procedure. "I wasted a whole afternoon trying that," I said. Colowick turned to Lowry and said with mock gravity: "Imagine, Ollie, he wasted a *whole* afternoon."

Imagination or hard work? At either extreme—speculating about complex phenomena or doggedly collecting data—success may come on occasion and draw acclaim. But the most consistent approach for acquiring a biochemical understanding of nature lies in between. The novel is yet to be written that captures the creative and artistic essence of scientific discoveries and dispels images of the scientist as dreamer, walking in the woods awaiting a flash of insight or of the scientist as engineer, at an instrument panel executing a precisely planned experiment. Some intermediate ground, hard work with a touch of fantasy, is what I have sought for myself and my students.

If asked to name varieties of mental torture, most scientists would place writing near the top of the list. As a result, scientific papers are usually put off or dashed off and demean the quality and value of the work they describe. Writing a paper is an integral part of the research and surely deserves the small fraction, say five percent, of the time spent finding the thing worth reporting. Yet, I feel uneasy seeing students and colleagues writing at their desks during "working hours" rather than busy at the laboratory bench. Whereas taking time to prepare a scientific report is unavoidable, writing a book always seemed an unconscionable abdication from research until I wrote one.

Writing *DNA Synthesis*, a 400-page book (80), was a surprise in many ways. First, the effort was far greater than I imagined. Very little from lecture notes and reprints could be lifted and placed in the right context and still remain readable. I was also surprised by the pleasure I found in reworking and polishing sentences and paragraphs for brevity and clarity, a satisfaction I had never found in crossword puzzles or other word games. Best of all, I could present my work, views, and excitement about the enzymology of DNA replication to an unexpectedly wide audience. The book—adopted as a text for some courses—became the reference source for writers of reviews of DNA replication and for authors of textbooks of biology and biochemistry.

The sequel to *DNA Synthesis*, entitled *DNA Replication* (23), came out in 1980. Twice the size of its predecessor, it was really a new book in scope and organization as well as in expanded contents. Despite its inflated size, it was a better book and found a wider readership. However, progress in this field is so rapid that revisions are needed annually. As an experiment in publishing, I assembled a 273-page "1982 Supplement to DNA Replication" (67), which extended the life of its parent. The publishers objected to "1982" in the title and correctly saw it as an advertisement of obsolescence. There have been no further supplements.

Having regarded teaching and book writing as deviant activities for a dedicated scientist, then surely the administrative work of a departmental chairman should be beyond the pale. Yet, I served as chairman for more than 20 years and never found it a serious intrusion on my time or attention. On the contrary, the benefits of creating and maintaining a collegial and stimulating scientific circle were well worth the investment I made. With excellent administrative assistance and the eager participation of my faculty colleagues, direction of departmental activities took no more time than being a conscientious member of the department.

Involvement in medical school and university affairs is a far different matter. I never found the skills and patience to function at these levels. For me, the most burdensome feature of being a departmental chairman was the obligated service on the Executive Committee of the Medical School, preoccupied with budgets, promotions, interdepartmental feuds, and salaries. In 6 years at Washington University and 10 at Stanford, I cannot recall a deliberate discussion of science or educational policy. No wonder I had no interest in being the dean of a medical or graduate school on occasions when this possibility was raised.

Increasingly conspicuous in current scientific life are the extramural administrative and educational activities, which, with the attendant travel, may consume half the time of prominent members of a science faculty. Lectures and visiting professorships, scientific meetings and society councils, government panels and advisory boards, consultantships in industry—all are prestigious, diverting, less demanding than research, and terribly tempting. I have done less than most, but have been unable to resist participating, particularly in writing essays (81–83), testifying for federal support of research and training, and most recently in the founding and development of a biotechnology enterprise (the DNAX Research Institute, Inc., later acquired by the Schering-Plough Corp.) with the mission of applying the techniques of molecular and cellular biology to the therapy of diseases of the immune system.

All these nonresearch activities, in and out of the university, fail to give me a deep sense of personal achievement. In research, it is up to me to select a

corner of the giant jigsaw puzzle of nature and then find and fit a missing piece. When after false starts and fumbling, a piece falls into place and provides clues for more, I take pleasure in having done something creative. By contrast, in my other activities, which are just as personal, all I do, it seems, is try to behave in a commonsensical, fair, and responsible way, as anyone else would. With research so dominant over my teaching, writing, and administrative activities, in sharply descending order of importance to me, I sometimes wonder whether, valued for their contributions to science, this order might be inverted.

Beginning with administration, consider the creation and management of the Stanford Biochemistry Department. It was started in St. Louis as the Microbiology Department. From there, Paul Berg, Bob Lehman, Dave Hogness, Dale Kaiser, and Mel Cohn moved with me to Stanford in 1959 to be joined by Buzz Baldwin and a few years later by George Stark and Lubert Stryer. In polls of peers, the Department has been accorded a top rating for many years, and is regarded as a major source of discoveries basic to recombinant DNA and the genetic engineering revolution. More than 500 people trained in the department now staff and direct departments of biochemistry and molecular biology all over the world. The organization and development of this notable faculty and its preservation, largely intact, against strong and attractive centrifugal forces, I would have to admit is a unique achievement.

As for writing, the monographs on DNA replication, with more than 40,000 copies sold, have made it easier for others to enter and work in this field. More than offering a readable account of a forbiddingly specialized area of biochemistry, these books have helped revive an appreciation that enzymology provides a direct route toward solving biologic problems and creates reagents for the analysis and synthesis of a great variety of compounds for all branches of biologic science.

With regard to teaching, assumption of credit for the success of a student has always puzzled me. There simply are no controls in these experiments. How do I know, given a motivated, gifted student, whether I have been a help or hindrance? Nevertheless, having involved myself in the daily scientific lives of my students, I may have guided some of them in directions that attract me and thereby diverted them from a career in biology or chemistry to the love and pursuit of biochemistry and enzymes. These progeny now include illustrious figures in science who have spread this gospel to a widening circle of "grandstudents" and "great-grandstudents."

Finally, even were I forced to agree that my activities in administration, writing, and teaching had a singular quality, I would have to concede further that my discoveries in science did not. Very likely, they would have been made by others soon after. Yet in the last analysis, I will argue that for me it

was the research that mattered most, because all my attitudes and activities were shaped by it.

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