In all organisms, genes coding for proteins are transcribed by the multisubunit complex, DNA-dependent RNA polymerase. In prokaryotes, this is accomplished by the core RNA polymerase linked to a number of sigma factors that specify its binding to different promoter elements. In eukaryotes, this process is more complex because of the presence of three distinct RNA polymerases, each responsible for the transcription of a different class of RNA. During the past few years, structural and biochemical studies have provided enormous insight into how these macromolecular machines interact with DNA and carry out many of the detailed steps in the initiation and elongation of RNA chains (1, 2). Today, DNA microarray experiments permit the simultaneous analysis of the transcription of thousands of genes in a single experiment under a variety of conditions. Because of the availability of data banks containing sequences of many genomes, we can change or delete promoter sites selectively to alter transcription. More than 50 years ago such techniques and knowledge were unimaginable. However, it was during the period spanning 1955–1961 that discoveries were made which resulted in the isolation of polynucleotides phosphorylase, *Escherichia coli* DNA polymerase I, and DNA-dependent RNA polymerase and the solution of the genetic code, all of which contributed importantly to the future of research. Recollections concerning the discovery of polynucleotide phosphorylase (3), DNA polymerase I (4), and a historical review of the deciphering of the genetic code (5) have appeared. The purpose of this article is to describe the discovery of DNA-dependent RNA polymerase. Because it is included among articles entitled “Reflections,” I have taken the liberty to describe this discovery as a personal journey and how I and others contributed to this important finding.

Early Training in Biochemistry

My early training was carried out in the Department of Biochemistry at Western Reserve University where I received my Ph.D. in 1953. The biochemistry department at that time was chaired by Harland G. Wood. The focus of the research effort of the department was primarily intermediary metabolism. Though all graduate students were required to take advanced chemistry, biochemistry, and microbiology courses, my exposure to what could be described as the beginnings of molecular biology was minimal and my appreciation of genetics was rudimentary at best. These deficiencies were personally troubling because my sister Zella was married to Salvador Luria. Dating back to 1945 and my first visit to the Cold Spring Harbor Biological laboratories where the Lurias were spending a sabbatical year, I had listened with little comprehension to Salva, Max Delbrück, Al Hershey, Renato Dulbecco, and other luminaries of the phage field discuss their research. During my early graduate training I noticed their disdain for biochemistry, and years
later, I was more than pleased when they contacted me for biochemical advice. In 1951, hoping to overcome this lack of knowledge, I took the phage course at Cold Spring Harbor. I recall being excited about the course and the phage field, but this enthusiasm was dissipated quickly on my return to Cleveland. My fellow students, who at that time included Sasha Englard, Paul Berg, and Harry Rudney, among others, were too engrossed in their own research efforts to listen to my description of the newly discovered area of lysogeny or my appreciation of the Luria-Delbrück fluctuation experiment. I recall Luria telling me during a visit to Urbana in the summer of 1952 that Jim Watson (who had been a graduate student in his laboratory at Indiana University) was carrying out important work on the structure of DNA. It made little impression on me since by that time I was immersed in studying the enzymatic phosphorylation of vitamin B₆ derivatives and their function as coenzymes. The excellent training we graduate students received in biochemistry at Western Reserve was influenced by Harland Wood’s work ethic and devotion to science. His extraordinary research accomplishments and role in training young scientists have been chronicled in wonderful articles by David Goldthwait and Richard Hanson (6) and Paul Berg (7). Wood invited many distinguished scientists of the period who presented exciting seminars that kept us up to date with their new discoveries. These included Hans Krebs, Fritz Lipmann, Ephraim Racker, Severo Ochoa, Fred Sanger, Arthur Kornberg, Seymour Cohen, Melvin Calvin, and Bernard Horecker. Because a substantial focus of the department was on CO₂ fixation, I became interested in photosynthesis. I was particularly excited by the seminars given by Calvin and Horecker. Between 1950 and 1952, Calvin’s group carried out in vivo studies on CO₂ fixation in photosynthetic organisms while Horecker’s laboratory examined the enzymes involved in the oxidation of 6-phosphogluconate (Warburg-Dickens-Lipmann pathway) in animal tissues and yeast. Independently, they both identified a number of key common phosphorylated sugar derivatives (ribulose phosphate in particular), suggesting that the pathway by which CO₂ fixation occurred in photosynthesis was biochemically tractable. I thought that the purification and isolation of the enzymes involved in photosynthetic CO₂ fixation was the best way to define this pathway. Because this was the approach Horecker’s group used to elucidate the oxidation of 6-phosphogluconate, which then led to the discovery of the transketolase and transaldolase, I decided to apply for postdoctoral training in his laboratory at the National Institutes of Health. Horecker graciously accepted me as a postdoctoral fellow. In the interim period, Wood convinced me that I needed additional training in chemistry. For that reason, immediately after receiving my Ph.D. degree, I spent 1953–1954 working in Albert Neuberger’s laboratory at the Institute for Medical Research at Mill Hill in London, England, where I carried out stereoc hemical organic syntheses. I was given the problem of synthesizing hydroxyornithine in order to evaluate whether it rather than hydroxyproline was a constituent of collagen. Though I never accomplished this feat, I did synthesize both D- and L-carnitine primarily because they could be readily made from D- and L-isoserine, well defined stereochemical intermediates I had prepared for the production of hydroxyornithine. I never published this work because I naively considered these compounds biologically uninteresting at the time. Shortly thereafter, carnitine was shown to function as a carrier of acetyl groups through the mitochondrial membrane (8, 9). However, this period did give me the opportunity to learn some organic chemistry, how to use the many volumes of Beilstein, and travel.

Postdoctoral Training at the NIH

Between 1954 and 1956, I was a postdoctoral fellow at the National Institutes of Health in Horecker’s laboratory. During this period, in collaboration with Bernie, Pauline (Polly) Smyrniotis, and Arthur Weissbach, we isolated and characterized a number of enzymes and substrates that firmly established the photosynthetic CO₂ fixation cycle proposed by Calvin, which Horecker has summarized in a “Reflections” article (10). Horecker, Ed Heath, and I also solved a vexing biochemical puzzle at that time, which involved the fermentation of pentoses by several Lactobacillus species whereby the methyl and carboxyl residues of acetate arise from the carbon atoms 1 and 2 of pentose, respectively, whereas lactate is derived from carbon atoms 3, 4, and 5. We demonstrated that xylulose 5-phosphate was cleaved phosphorolytically to acetyl phosphate and triose phosphate by a thiamine pyrophosphate-dependent enzyme that we called phosphoketolase (11). This highly productive period was due largely to Horecker’s impressive expertise in carbohydrate metabolism, enthusiasm for research, and general positive personality. Harry Eagle, also cognizant of this buoyant demeanor, aptly described Bernie as a reincarnated Dr. Pangloss. The exploits of the Enzyme Section, first organized by Arthur Kornberg and headed by Horecker during the mid-1950s, included a daily luncheon seminar on literature (and any new exciting findings) with Herbert Tabor, Celia Tabor, Alan Mehler, Leon Heppel, Herman Kalckar, Jesse Rabinowitz, Bruce Ames, and anyone else willing to squeeze into the small library of Building 3 or later on the 9th floor of the Clinical
Center and have been described in a number of “Reflections” articles (10, 12). During this period, I recall Severo Ochoa’s visit at which time he was scheduled to present a seminar on aspects of oxidative phosphorylation in Azotobacter vinelandii. Though he initiated his seminar discussing \( \beta \)-hydroxybutyrate oxidation, I recall being tremendously excited when he described the experiments that he and Marianne Grunberg-Manago had carried out, which led to the discovery of polynucleotide phosphorylase (PNPase). At that time, Ochoa elicited Leon Heppel’s expertise to help characterize the polymer products. Leon, in collaboration with Roy Markham’s group at the Molteno Institute in Cambridge, England, pioneered many of the chemical and enzymological techniques that established the 5’-3’ internucleotidic phosphodiester linkage in RNA. During his studies with the A. vinelandii PNPase, Heppel noted that the preparations were contaminated with an activity that converted ADP to ribose phosphate and adenine, which severely limited the synthesis of poly(A). He proposed that Horecker, he, and I jointly investigate this problem. We quickly showed that it was due to an enzyme that cleaved AMP to adenine and ribose 5-phosphate. The PNPase preparations also contained a myokinase activity that converted ADP to AMP and ATP. Surprisingly, the cleavage of AMP required ATP only as an effector (13). During these studies, I delighted in my interactions with Leon. These included his many handwritten notes (sometime 5–10 pages), which often included totally “off the wall” quotes from newspapers, information about various classical symphonies, and quizzes. During our work on the AMP-cleaving activity, he once appeared in the doorway of my laboratory in Building 10 asking what he should do next. When I responded that I thought that he should think more about what we were doing, he quickly stated “I don’t have the time. Just tell me what to do.” I suspect that these pranks provided Heppel periods of respite from his focused and dedicated research efforts that resulted in his many important contributions to RNA biochemistry, already described by Maxine Singer (12).

**Early Years in St. Louis and Studies in Nucleic Acid Biochemistry**

Though I thoroughly enjoyed my postdoctoral period at the NIH, in 1956 I joined the Microbiology Department of Washington University in St. Louis, MO, which was chaired by Arthur Kornberg. My decision to leave the NIH was influenced in part by my strong aversion to the loyalty oath required at government institutions and more so by my desire to return to a more academic environment and interests in studying nucleic acid biochemistry. Kornberg’s research accomplishments were already legendary, and the department he had assembled in St. Louis was first rate and included Paul Berg, Melvin Cohn, David Hogness, and Dale Kaiser. At that time, I. R. (Bob) Lehman was a postdoctoral fellow in Kornberg’s laboratory and was appointed to the faculty soon after. The group was highly congenial, mutually supportive, and intensely research oriented. The department was housed on the fourth floor of an ancient clinic building that bustled with the influx and efflux of many patients. A rickety old caged elevator carried us to our laboratories on the fourth floor. As the elevator ascended the patients milling about in the waiting rooms became more visible because of the high ceilings of the large waiting rooms. On one occasion while riding in the elevator with Kornberg, he told me that he felt exhilarated by the clinic because it kept him in touch with reality. Years later, I told him that I was impressed by his positive attitude about the old clinic building at Washington University. He looked at me with horror and said, “I thought it was terrible.”

During my postdoctoral period at the NIH and our interests in pentose metabolism, Horecker and I wondered how deoxyribose was formed but made no effort to investigate this problem. Earlier studies by Ephraim Racker (14) demonstrated that deoxyribose 5-phosphate could be formed by an aldol condensation of glyceraldehyde 3-phosphate and acetaldehyde, a reaction catalyzed by the enzyme deoxyribose-phosphate aldolase. This enzyme was found widely distributed in microorganisms and animal tissues. However, its physiological role as the source of deoxyribose in DNA was questioned by the findings of I. A. Rose and B. S. Schweigert (15), who demonstrated that uniformly labeled ribonucleosides (isolated from Euglena grown on \( ^{14} \)CO\(_2\)) were converted into deoxynucleotides of DNA in vivo with no change in the ratio of the radioactivity found in the base and sugar. Impressively, these results were obtained with cytidine in *E. coli* and the rat as well as with GMP and CMP in *Lactobacillus leichmanii*. I decided to study whether ribonucleotides were reduced directly to deoxynucleotides when I got to St. Louis.

However, soon after I arrived in St. Louis, Harland Wood contacted me and wanted to know whether the glucose fermentation pathway used by *Leuconostoc mesenteroides* included the phosphorolytic cleavage of pentose that Horecker, Heath, and I had discovered. His laboratory had successfully used the pattern of C\(^{14}\) in the glucose of liver glycogen as an indicator of carbohydrate metabolic pathways (16). They showed that glucose fermentation by *L. mesenteroides* produced equimolar quantities of CO\(_2\), ethanol, and lactate from the carbon atoms 1,
2 and 3 and 4–6 of glucose, respectively. Because the reagents required to detect phosphoketolase were readily available (albeit in Horecker’s freezers at the NIH), I had little difficulty in showing that xylulose 5-phosphate was phosphorolyzed by extracts of E. mesenteroides to acetate and triose phosphate (17), verifying the fermentation pathway which made the organism such a useful means for the isolation of each glucose carbon atom. Upon completion of this diversion, I began studies on the reduction of ribonucleotides to deoxynucleotides. At this time, a highly sensitive colorimetric assay for deoxyribose appeared (16).

It depended on the periodate oxidation of the sugar to malondialdehyde, which on interaction with thiobarbituric acid yielded a chromogen with an absorption maximum at 532 μm. Incubation of AMP or ribose 5-phosphate with extracts of E. coli resulted in substantial levels of the chromogen. However, the spectrum of the product formed in the periodate-thiobarbituric acid test differed from that found with deoxyribose. Arthur Weissbach, who was then working at the NIH, made similar observations, and we combined our efforts to identify the product. In a short time, using chemical and enzymatic approaches, we showed the product was 2-keto-3-deoxyheptonic acid, an intermediate in shikimic acid formation, which was identified as 2-keto-3-deoxy-6-phosphogluconic acid. Bucher and co-workers found the expected 3-deoxy-6-phosphogluconic acid oxidation product, and we combined our efforts to identify the product. In a short time, using chemical and enzymatic approaches, we showed the product was 2-keto-3-deoxyheptonic acid, an intermediate in shikimic acid formation, which was identified as 2-keto-3-deoxy-6-phosphogluconic acid, and then converted to polyphosphoric acid at 250 °C, neutralized, and then incubated with cytidine at 60 °C for 3–4 h. This resulted in the formation of a mixture of labeled 2′-, 3′-, and 5′-CMP from which 5′-CMP could be isolated. I then found that the incubation of 32P-labeled 5′-CMP with crude extracts of E. coli resulted in the production of low levels of 32P-labeled 5′-dCMP, which were resolved from 32P-labeled 5′-CMP by borate-paper chromatography. I noted that the reaction required ATP and that treatment of reaction mixtures with potato apyrase (which cleaved the β and γ phosphate residues of NTPs) increased the yield of [32P]dCMP. My next task was to purify the system and identify the reducing agent. At this point, I made a fateful decision to change the paper chromatographic assay for a more rapid one. Kornberg’s laboratory (which at that time included Maurice Bessman, Bob Lehman, and Ernie Simms) was examining the action of E. coli DNA polymerase I, first discovered by Arthur’s group in 1955. Their exquisite work had shown that purified enzyme fractions incorporated all 4 dNTPs into DNA and (most critical for the assay I planned to use) worked specifically with dNTPs and not rNTPs. I decided to couple the [32P]CMP conversion to [32P]dCMP reaction with the DNA polymerase I system and measure the incorporation of dCMP into DNA. Acid precipitation was a more rapid means to measure this reaction. For this purpose, I incubated [32P]CMP with crude extracts of E. coli (which contained kinases capable of forming the nucleoside di- and triphosphate derivatives) and then added dATP, dGTP, dTTP, and purified DNA polymerase I. Indeed labeled DNA accumulated. The reaction required crude extracts, the dNTPs, and DNA and was blocked by the addition of pancreatic DNase. Initial attempts to further purify the components necessary to support the incorporation of 5′-32P-labeled CMP into DNA resulted in considerable loss of activity. Searching for factors that increased the yield, I discovered that a mixture of Mn2+ + Mg2+ was considerably more effective than Mg2+ alone. Much to my surprise, when I isolated the labeled DNA products in the reaction and degraded this material with pancreatic DNase I plus venom phosphodiesterase, conditions that I expected would yield 32P-labeled 5′-dCMP, I observed that the labeled CMP had been incorporated directly into DNA with no detectable labeled dCMP present in DNA. The 32P-labeled polynucleotide product was rendered partially acid-soluble after either RNase or NaOH hydrolysis but completely acid-soluble by DNase I digestion. These properties suggested that rCMP had been incorporated distributively throughout the polynucleotide chain synthesized. Reactions that were carried out with [α-32P]CTP + UTP + GTP + ATP (no dNTPs) yielded low levels of labeled acid-insoluble mate-
rials, which were reduced upon omission of a single rNTP. I published this work in a paper entitled “The Enzymatic Incorporation of Ribonucleotides in Polydeoxynucleotide Material” in which I indicated that the biological significance of this enzymatic reaction was unclear (22). Nearly 5 years later, it was shown that the presence of Mn$^{2+}$ altered the nucleotide specificity of DNA polymerase I, leading to the incorporation of both rNTPs and dNTPs into DNA (23). In retrospect, it is likely that my findings were due to multiple activities including DNA polymerase I and possibly low levels of DNA-dependent RNA polymerase. How I missed discovering RNA polymerase during these studies in 1958 mystifies me to this day. Though my initial goal was to discover how deoxynucleotides were formed from ribonucleotides, I never returned to this problem. In 1961, Peter Reichard’s laboratory demonstrated that rCDP was converted to dCDP by extracts of E.coli (24) and later showed that the reducing agent was a pair of intrinsic sulfhydryl groups on the enzyme (ribonucleotide reductase) that was regenerated after catalyzing the reduction by thioredoxin, which acts as a protein disulfide reductase (25).

The Discovery of DNA-dependent RNA Polymerase

In 1958, I moved to New York and joined the Microbiology Department at New York University, School of Medicine. Bernie Horecker was appointed chairman of the department. At that time, influenced by the work I carried out in St. Louis, I decided to focus on RNA synthesis. During this period, our thinking about the biosynthesis of RNA was dominated by the action of PNPase first discovered by Grunberg-Manago and Ochoa (3) and independently somewhat later by Uriel Littauer when he was a post-doctoral fellow in Arthur Kornberg’s laboratory (26). The role of PNPase in synthesizing biologically relevant RNA, however, was questioned by its mode of action. The enzyme required high levels of ribonucleoside diphosphates as substrate and produced P$_i$ and high molecular weight RNA polymers whose composition depended on the level and particular nucleoside diphosphates added to the reaction. The reaction was freely reversible, and RNA polymers were readily phosphorolyzed to the corresponding ribonucleoside diphosphates. There was no evidence of a template-directed synthesis of RNA, though at the time of its discovery the possibility was raised that the aberrant products formed resulted from the loss of factors during its purification that were required to form more physiological RNA products. Though further studies validated these concerns and PNPase was found to be a variable component of the prokaryotic degradosome, which regulates RNA hydrolysis, the enzyme proved to be of enormous importance. It provided the means for the synthesis of many different RNAs that helped to define the genetic code and the first hybridization experiments between poly(A) and poly(U), carried out by the laboratories of Alex Rich and Robert Warner (27, 28) and between RNA and DNA (poly(A) and a synthetic poly(dT) oligomer chemically synthesized by Khorana) by Alex Rich (29). Marmur, Doty, and their colleagues also showed that complementary single-stranded DNAs formed by heat denaturation of duplex DNA could be reannealed to duplex DNA by slow cooling (30). These discoveries eventually led to the demonstration that all RNA species were formed from DNA and later contributed to the discovery of RNA splicing.

During the period between 1953 and 1958, a number of reports described the existence of enzyme activities that utilized rNTPs rather than rNPs and extended RNA chains by only a few nucleotides (specifically C and/or A residues). They were defined eventually by the RNA terminal -CCA adding enzyme, an activity dedicated solely to the addition of C and A residues to the 3’-ends of tRNAs (31, 32). Subsequently, it was shown that DNA sequences encoding tRNAs do not include the 3’-terminal -CCA sequence, which is essential for amino acid acylation. Uniquely, in 1960, Edmonds and Abrams reported the synthesis of poly(A) chains from ATP (33), the first indication of poly(A) polymerase. At this time, it was evident that none of the aforementioned enzyme activities explained how RNA was synthesized. In contrast to the biosynthesis of DNA, which simplistically could be considered a single entity, the biosynthesis of RNA appeared to be more complicated because of its heterogeneity. Furthermore, there was evidence that the function of these distinct RNA species differed. It was evident that a number of specific small molecular weight RNAs (tRNAs) were carriers for individual amino acids in protein synthesis (34, 35) whereas the multiple larger RNA species (ribosomal RNAs), constituting the bulk of cellular RNA, existed as a ribonucleoprotein complex where amino acids appeared to be incorporated into proteins (36). By the late 1950s, it was generally accepted though not proven that the genetic information coded in the sequence of DNA contributed to the amino acid sequence of proteins and RNA acted as an intermediate in this process. In support of this notion, the pioneering phage work done by Seymour Cohen showed that infection of E.coli by virulent phages such as T2 caused bacterial DNA degradation that blocked bacterial protein and DNA synthesis and diverted the host to synthesis phage DNA and phage proteins (37). Similar phage experiments by AI Hershey noted that a minor RNA spe-
cies was rapidly synthesized and degraded (38). By far the most compelling evidence for an important role for DNA in RNA synthesis came from the experiments of Volkin and Astrachan in which T2 infection of E. coli in the presence of $^{32}$P, resulted in the rapid formation and degradation of RNA containing the same base ratio of the T2 DNA (39). Though I was fully aware of these results and had thought about the role of DNA in RNA synthesis in St. Louis, my efforts to screen for DNA-dependent RNA synthesis started in 1959 and were carried out with E. coli extracts. In light of my earlier confusing work that detected the incorporation of ribonucleotides into DNA, I decided it was essential to uncover an enzymatic activity that required DNA and all four rNTPs. Influenced by the findings of the Kornberg laboratory that a single enzyme carried out DNA synthesis in the presence of a DNA template and all four dNTPs, I thought likely that a single enzyme dedicated to the synthesis of RNA existed. To avoid complications from the tRNA -CCA terminal adding enzyme, I used [$\alpha-^{32}$P]UTP and carried out experiments with E. coli DNA to circumvent the possibility that there might be species specificity.

Results from our initial experiments were encouraging, but crude extracts from E. coli were variable, especially in their dependence on exogenously added DNA. Attempts to remove DNA resulted in considerable loss or inactivation of the activity. After much effort, I found that protamine sulfate co-precipitated the ribonucleotide incorporating activity, which could be extracted preferentially at low salt concentrations. Subsequent purification steps yielded preparations that supported E. coli DNA-dependent ribonucleotide incorporation. The possibility that this activity was because of PNPase I thought remote but necessary to rule out because Littauer and Kornberg (26) had shown that E. coli was a rich source of this enzyme. Much to my discomfort, we readily detected its presence in our preparations. I was encouraged to think that the DNA-dependent reaction was not catalyzed by PNPase because the concentration of rNTPs required to support robust DNA-dependent RNA synthesis was in the range of $10^{-5}$ M whereas $10^{-3}$ M quantities of rNDPs were required for PNPase activity. Furthermore, RNA could not replace DNA under the conditions used, and the presence of P, did not block the DNA-dependent synthesis of RNA but markedly inhibited the PNPase activity.

In 1959, Samuel B. Weiss’ laboratory reported that rat liver nuclei supported RNA synthesis in reaction mixtures containing all four rNTPs (40). Importantly, the omission of a single rNTP markedly reduced RNA synthesis. They also showed that the synthesis of RNA was sensitive to RNase and that alkaline hydrolysis of the labeled RNA product led to the recovery of radioactivity in all four 2’ (3’)-mononucleotides. However, the addition of DNase reduced the incorporation of ribonucleotides into RNA only slightly. Clearly, Weiss’ group had detected a RNA-synthesizing system that differed from the tRNA -CCA terminal adding enzyme, but at that time it was not clear whether they were examining a DNA-dependent reaction. By the late spring of 1960, we had reproducibly demonstrated a marked stimulation of RNA synthesis by externally added DNA with our E. coli preparations. The synthesis of RNA was completely blocked by low levels of DNase or RNase and required all four rNTPs. Importantly, a wide variety of heterologous DNAs supported RNA synthesis. We published a note describing these findings (41).

Much to my surprise, in the same issue of the journal containing our results, Audrey Stevens, who was then working as a postdoctoral fellow in Leon Heppel’s laboratory at the NIH, reported that extracts of E. coli incorporated labeled ATP into RNA in a reaction requiring all four rNTPs (42). Her paper indicated that the reaction was RNase-sensitive but did not evaluate the role of DNA in the reaction (42). In a later issue of the same volume of this Journal, James Bonner’s group at the California Institute of Technology reported similar findings with extracts of peas (43). Thus, RNA synthesis was clearly becoming a highly competitive race.

Though we had used only partially purified fractions in our experiments, I noticed that the incorporations of ribonucleotides mirrored the base composition of the DNA template used. To study this in more detail, we compared DNAs that had high and low AT contents (varying as much as 4-fold) as templates in the reaction and showed that the pattern of ribonucleotide incorporation reflected these biases. During this period, I contacted Erwin Chargaff at Columbia University hoping to obtain a small amount of mycobacterial DNA (high GC content). Chargaff required that I inform him in person rather than by telephone how I planned to use his DNA. Shortly thereafter, I went to his laboratory and summarized the experiments I planned to carry out with his DNA. I began by telling him that I was studying a system in which DNA appeared to act as a template for RNA synthesis. He required that I define what I meant by DNA and what I meant by RNA. After much effort on my part, he appeared impressed by my results and agreed to give me the DNA. I followed him into his laboratory where he presented me with a few milligrams of the DNA from a bottle stored on a chemical shelf at room temperature. Somewhat surprised at seeing DNA stored at room temperature, I apol-
ogetically asked whether he had any material stored at a lower temperature. He did not. Because the assay used to measure DNA-dependent RNA synthesis was rapid, within a short time after returning to my laboratory I realized that this DNA preparation was inactive because it was quantitatively acid-soluble. I thought little about this experiment, but I was amused years later when I read a short article written by Maurice Wilkens in which he described his early experiments on the structure of DNA. At that time he wanted to determine whether the x-ray diffraction pattern of DNA was influenced by its base composition and carried out such studies with mycobacterial DNA obtained from Erwin Chargaff. To his disappointment, he found no diffraction with this preparation. I suspect that Wilkens and I did experiments with the same material.

By the Spring of 1961, in collaboration with John J. Furth who as a fellow in the Pathology Department joined my laboratory as a postdoctoral fellow, we had accumulated data to conclude that the RNA synthesized by DNA-dependent RNA polymerase reflected both the base composition as well as the sequence of the DNA template used in the reaction. RNA synthesis carried out in the presence of two special DNA templates supported this conclusion. They included the use of a short poly(dT) oligomer chemically synthesized by Gobind Khorana’s group (and generously supplied by him), which resulted in the synthesis of poly(A) without the incorporation of any other rNTP and the demonstration that a poly[d(A-T)] copolymer containing the alternating ATAT . . . sequence directed RNA synthesis only with ATP and UTP in the presence or absence of GTP and CTP. More importantly, the AUAAU . . . sequence (32). Poly[d(A-T)] was first discovered in Arthur Kornberg’s laboratory and occurred in an apparently unprimed reaction catalyzed by DNA polymerase I. It was discovered in 1957 by Howard Schachman (during a sabbatical in Arthur’s laboratory) and Bob Lehman. The discovery of this polymer has been described by Lehman (4). We obtained poly[d(A-T)] from Arthur Kornberg. When I called Arthur to request this material, he informed me that Michael Chamberlin, then a graduate student in Paul Berg’s laboratory, was also working on E. coli RNA polymerase (unknown to me) and planned to do the same experiment with this DNA and felt obliged to give it to him first. Arthur proposed that after Mike did the experiment, he would consider sending the material to me. Begrudgingly, I understood this collegial requirement. Shortly thereafter, Arthur informed me that Chamberlin found that the poly[d(A-T)] copolymer did not support RNA synthesis and if I still wanted the material he would send it to me. Because Mike’s experiment yielded a negative result, I said that I would still like to try the experiment. I recall that we later exchanged enzymes and discovered that the early preparations he made were contaminated with more nuclease activity than ours, possibly explaining why his experiment failed. Among his many important contributions to the function of DNA-dependent RNA polymerase, in 1970 Chamberlin discovered that infection of E. coli by phage T7 induced the formation of a single polypeptide RNA polymerase (44). I was surprised by this finding because we had carried out similar experiments but failed to detect this activity. When I told him of our failure, he commented wryly that you win some and you lose some.

At this time, Weiss and Nakamoto reported the isolation of DNA-dependent RNA polymerase from Micrococcus lysodeikticus (45). In contrast to their earlier studies with rat liver nuclei, RNA synthesis with the purified M. lysodeikticus enzyme was completely dependent on DNA and carried out extensive RNA synthesis that possessed the same frequency of dinucleotide pairs (nearest neighbor analysis) as that found in DNA templates (46). Subsequent experiments from the laboratories of Stevens (47) and Chamberlin and Berg (48) showed that the molar amount of nucleotides incorporated into RNA by RNA polymerase reactions exceeded the molar level of nucleotides added as DNA, indicating that the DNA template acted catalytically. Experiments carried out by Geidushek, Nakamoto, and Weiss (49) and later by Chamberlin, Baldwin, and Berg (50) demonstrated that RNA products generated in vitro with purified RNA polymerase from duplex DNA were not associated with template DNA and formed DNA-RNA hybrids specifically with the DNA used in the reaction after heating and cooling. Importantly, both laboratories showed that the template duplex remained intact after supporting extensive RNA synthesis. These findings established that RNA polymerase copied duplex DNA in a fully conservative manner.

1961 Cold Spring Harbor Symposia on Quantitative Biology

In my opinion, this early phase (1959–1961) concerned with the discovery of DNA-dependent RNA polymerase culminated with the 1961 Cold Spring Harbor Symposia on Quantitative Biology, which was entitled “Cellular Regulatory Mechanisms.” The highlight of the meeting was the paper of François Jacob and Jacques Monod on the “Regulation of Gene Activity” in which they proposed the “messenger” RNA hypothesis to explain the role of unstable RNA synthesized after phage infection with the wealth
of information they and their co-workers had gathered on the kinetics of β-galactosidase induction and repression in bacteria (51). Their paper masterfully defined and predicted transcriptional regulation. It included schemes for the control of protein synthesis through the negative action of trans-acting regulatory proteins (repressors, later shown to be positive as well), target sites at which repressors act (called operators), and the critical need for rapidly turning over “messenger” RNAs arising as intermediates from DNA that program the protein-synthesizing machinery located in ribosomes. Other exciting papers presented at the meeting by Sol Spiegelman (52) and François Gros et al. (53) contributed importantly to the “messenger” RNA hypothesis. Their data extended the Volkin-Astrachan findings and included the isolation of phage T2 RNA as a physical entity, the demonstration that it possessed the same base ratio as T2 phage DNA, hybridized specifically to T2 DNA, and was ribosome-bound with a linkage more sensitive to disruption than normal ribosomal RNA. In keeping with the universality of the “messenger” RNA model, they also detected the synthesis of metabolically unstable RNA complementary to DNA in a variety of microorganisms. I presented a summary of our studies with the E. coli RNA polymerase, which provided an enzymatic mechanism by which DNA supported RNA synthesis (54). Sidney Brenner summarized the data that laid to rest the notion that ribosomal RNA present in ribosomes carried information for the synthesis of proteins and showed that T4 phage messenger RNA interacted with ribosomes and acted as the template for the synthesis of protein (55).

During the meeting, papers from the laboratories of Charles Yanofsky, George Streisinger, and Cyrus Levinthal focused on the gene-protein colinearity problem. Though I do not recall specific discussions about the genetic code, it was obvious that they planned to use a variety of lengthy strategies aimed at this solution. It was during this period, but unknown to me and most of us at the meeting, that Marshall Nirenberg and Heinrich Matthei at the National Institutes of Health carried out their dramatic experiments that would lead to the solution of the genetic code. In the early summer of 1961 I first heard of their work from Max Delbrück, who was at Cold Spring Harbor Laboratories. He called to find out whether I knew of the work carried out by Nirenberg and Matthei. Matthei was a student in the bacteriophage course at that time and like all students who participated in the course (as I had done in 1951) presented a short summary of his research efforts carried out at his home institution. Max summarized their results and asked if I believed them. My first response was no but I told him that I could check it out. I immediately telephoned Gordon Tompkins who I knew well and who was in the same group at the NIH as Nirenberg, and he told me that it was true. A historical review of the period describing this discovery has been presented by Nirenberg (5).

Epilogue

The important discoveries made between 1955 and 1961, like all important findings, raised many more questions than answers. They ushered in the fields of replication, transcription, and translation, their interdependence, and the complicated controls that govern cell cycle events. These early discoveries wedded biochemistry and genetics giving rise to molecular biology. The discovery of DNA-dependent RNA polymerase, simultaneously made by Sam Weiss, Audrey Stevens, and my own laboratory, was an exciting and stimulating period. It convinced me that I could do important work even if it required fierce competition. However, it also made me realize that if we had not made these discoveries, others would have soon after.

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