Synthetic RNA and the Poly-U Experiments, 1959-1961

Marshall W. Nirenberg is best known for his work on deciphering the genetic code by discovering the unique code words for the twenty major amino acids that make-up DNA, for which he won the Nobel Prize in Medicine or Physiology in 1968.

Nirenberg was the first government scientist to win the Nobel Prize. The National Library of Medicine and the Office of NIH History has amassed a collection of correspondence, laboratory administrative and research materials, and publications that documents Nirenberg's career as a researcher in biochemical genetics at the National Institutes of Health.

Dr. Nirenberg is featured in The Profiles in Science web site of the National Library of Medicine celebrates twentieth-century leaders in biomedical research and public health. Students appreciate the history, and share some of the excitement of early scientific discoveries in molecular biology. The National Library of Medicine is digitizing and making available over the World Wide Web a selection of the Marshall W. Nirenberg Papers, for use by educators and researchers.

In 2007, the Archives and Modern Manuscripts Program, History of Medicine Division completed a Finding Aid to the Marshall W. Nirenberg Papers, 1937-2003 (bulk 1957-1997). Individuals interested in conducting research in the Marshall W. Nirenberg Papers are invited to contact the National Library of Medicine.

The NLM digital materials and references provide the background for the series of six interviews conducted with Marshall W. Nirenberg, Ph.D., by Ruth Roy Harris, Ph.D., between September 20, 1995 and January 24, 1996.

The "Harris Interviews" took place in Nirenberg's laboratory on the campus of the National Institutes of Health (NIH) in Bethesda, Maryland. Harris also conducted several supplemental interviews, both by telephone and in person, with individuals either involved in the breaking of the genetic code or personally acquainted with Nirenberg: James Pittman, Joan Geiger, Philip Leder, Thomas Caskey, Sidney Udenfriend, and Perola Nirenberg. Interviews with Pittman and Geiger are now in the Marshall Nirenberg Collection at the National Library of Medicine (NLM). Notes from other interviews are held at the Office of NIH History.

A number of individuals and institutions worked on editing the interviews for clarity and content: Sarah Leavitt, Victoria Harden, Caroline Hannaway, Alan Schechter, Robert Balaban, and Alan Peterkofsky. Caroline Leake, Katrina Blair, and Mary Alvarez provided administrative and technical assistance. In 2008, Deborah Kraut edited and formatted the interviews to correspond to the NLM digital materials.

Each Section begins with the NLM digital summaries summaries and references. Additional references, when appropriate are added:

Nirenberg, Marshall W. Marshall W. Nirenberg Papers. 1937-2003. Located in: Modern Manuscripts Collection, History of Medicine Division, National Library of Medicine, Bethesda, MD; MS C 566.
http://www.nlm.nih.gov/hmd/manuscripts/ead/nirenberg566.html

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From NIH Profiles in Science:

http://profiles.nlm.nih.gov/JJ/Views/Exhibit/narrative/syntheticrna.html

Scientific understanding of the molecular basis of life increased dramatically after Oswald T. Avery's 1944 discovery that deoxyribonucleic acid (DNA) was the "transforming principle" and Francis Crick and James Watson's 1953 description of DNA's "double helix" structure. The process, however, by which DNA replicates itself during cellular reproduction, or how DNA expresses its genetic information, was still a mystery in the late 1950s. Many scientists theorized that the answer lay in the interaction between DNA and ribonucleic acid (RNA), but how this occurred, or how RNA translated information from DNA, was still then unknown. Such questions were very much in vogue at the time among both bacterial geneticists such as Joshua Lederberg" and protein chemists such as Christian Anfinsen.

In 1959, the RNA question also piqued Marshall Nirenberg's intellectual curiosity. Like formally trained geneticists, Nirenberg wanted to know if RNA was the chemical liaison or "messenger" between DNA, the genetic material, and proteins, the building blocks of cells. Nirenberg, however, had no formal training in molecular genetics. He had only attended evening courses about genetics, intended for scientists at the National Institutes of Health who were interested in conducting interdisciplinary research. Many of Nirenberg's colleagues felt that it was naive for a biochemist untrained in molecular genetics to commence a brand-new area of research; at least one felt that Nirenberg was committing "professional suicide."

In 1960, Nirenberg was joined by J. Heinrich Matthaei, a postdoctoral researcher from the University of Bonn in Germany then studying at Cornell University. They studied nucleotides–adenosine, cytosine, guanine, and thymine–the basic molecular units that are strung together to form long linear molecules of DNA and (with uracil substituted for thymine) RNA. Following recent work by the Swiss geneticist Alfred Tissieres, Nirenberg and Matthaei set out to create a "cell-free environment" that would let them examine how the minute mechanisms of RNA actually work, free of the normal biological processes of cells that could obscure molecular activities. After working with several possible organisms to serve as models, they settled on *Escherichia coli*, a common bacterium found in abundance in the human colon.

Nirenberg and Matthaei created a synthetic RNA molecule outside the bacterium and introduced this RNA to E. coli. They found that their synthetic RNA specified that phenylalanine, an amino acid, be added to the end of a growing strand of linked amino acids, the precursor to proteins. Nirenberg and Matthaei concluded that

traces of uracil had directed the synthesis of phenylalanine. On the RNA strand, synthetic RNA made of multiple batches of three units of uracil directed an amino acid chain composed entirely of phenylalanine. One three-unit batch of uracil could be read as UUU (poly-U), which was a three-letter shorthand method or "code word" for identifying phenylalanine. Nirenberg and Matthaei quickly realized that this was the messenger that they had been looking for. Their experiments proved that "messenger RNA," which transcribes genetic information from DNA, directs protein synthesis. That is, messenger RNA transmits the DNA messages that prescribe the assembly of amino acids into the complex proteins that drive living processes.
PROBLEMS IN THIS NEEDS REVISION

In August 1961, Nirenberg and Matthaei published their now-classic essay, "The Dependence of Cell-Free Protein Synthesis in E. Coli upon Naturally Occurring or Synthetic Polyribonucleotides," in the *Proceedings of the National Academy of Sciences*.

1995 – 1996 Harris Interviews

Marshall Nirenberg (MN): One night, ***shortly after I became an independent investigator***), I walked in the corridor adjacent to my lab and saw Bruce Ames, who was then one of the best scientists at the NIH. He was working at night in his lab, so on a whim I decided to ask his advice. I told him what my plans were as an independent investigator to determine whether ***mRNA (template? RNA*)** is required for cell-free protein synthesis of a protein. When I told him this, he looked at me. He said, “It's suicidal to do it.” So we both agreed that it would be extremely difficult and dangerous to work on this problem.[1]

I would like to emphasize that I could have done the work that I did only at the NIH. I couldn't have done it at a university. I would never have been awarded a grant to do the work because I had no experience in the field. That was a total turn-off right there and nothing to recommend my chances in the field. It was such far-out work, and it was in

such a highly competitive field that nobody would have given me a grant to do it. The fact is that I did it at the NIH. The NIH is the only place where I could have done the work because I didn't have to write a grant proposal here.

But, over the years things have changed at the NIH so that now you have to write very detailed reports on what you have done and what you propose to do. Always before there was an evaluation of the work individuals did. Now you have to write what you propose to do as well as what you've already done. That is a big change for the NIH, a change, I think, for the worse. One of the advantages of the NIH, I have always thought, is that you can change fields whenever you want to without trying to get a grant in the new field, which is virtually impossible to do. I have changed fields on two or three occasions, or even four occasions, and I always thought it was a tremendous advantage to be at the NIH because it was possible to do this.

Ruth Harris (RH): Could you please give the background for your use or development of the cell-free system that you used to study protein synthesis?

MN: Yes. This was largely based on the work of Paul Zamecnik and his colleagues. Zamecnik was the leader in protein synthesis, so I used his and his colleagues' techniques predominantly, their cell-free systems.[2] We had all the goodies that were needed for cell-free protein synthesis: an ATP generating system with phosphoenolpyruvate (PEP) that catalyzed the phosphorylation—that combination was the substrate from which phosphate was used to synthesize ATP, and cell extracts—magnesium.

I used their assay for amino acid incorporation into protein. It was extremely laborious with washing and repetitively centrifuging samples, pellets, and the pelleted protein, and then re-suspending and washing it with fresh TCA, trichloracetic acid. It took two days to do the experiment, mostly because of the repetitive centrifugations and the washing. I simplified the assay tremendously, and that was important.

At that time, in 1960, the work was getting very exciting. I don't think I took any vacations. I may have gone for a weekend to the beach, but I don't remember any vacation. That was my life. I just worked: that was all. I went out on dates and to parties, but this was what my life was.

RH: What were the questions you were asking at the outset of this work?

MN: Of course, it was a broader approach (than deciphering the code). It was actually ***a different set of questions***. Basically, we discovered messenger RNA. We found a fraction of it, and I called it template RNA at the time. What we did was to show that template RNA stimulated amino acid incorporation into protein. This was the first time that template RNA had ever been used. Concurrently, or maybe even a little bit after our work as done, [François] Jacob and [Jacques] Monod predicted the existence of template RNA simply by working with intact bacteria.[3]

Our work was the first work that had been done which showed that messenger RNA ***(mRNA)*** existed. We found a cell-free assay for messenger RNA. It was a new class of

RNA. The only other two classes that were known were ribosomal RNA and a very small transfer RNA, soluble RNA. Here we found an RNA that was a template for amino acid incorporation into protein. I rounded up as many different kinds of synthetic polynucleotides as I could and devised and wrote down experiments that would test their ability to serve as templates for protein synthesis.

RH: I am very curious about something that I have found in your journals. You have said elsewhere that Jacob and Monod coined the term “messenger RNA” in their 1961 article. But I found in your journal, on December 20, 1960, that you used the term.

MN: Really? I thought that they had coined the term “messenger RNA.” The term I used primarily, I thought, was “template RNA.” It is possible that Jacob and Monod sent Gordon Tomkins the preprint of the article. I didn't have any contact directly with them, but Gordon Tomkins received papers and preprints from many people, I think, including from them. I can just see in my mind’s eye Gordon coming in the lab with a paper, with a manuscript that he had received in the mail from somebody and just kind of slouching up against the refrigerator and saying, “Do you want to hear about a beautiful experiment?” I don't remember the paper that well. But they may have very well sent him a copy of a draft or the preprint of the manuscript.

Once we found that an RNA fraction stimulated amino acid incorporation into protein, we had discovered mRNA. We had discovered a means of assaying mRNA. The most obvious thing to do was to try as many different kinds of RNA as we could lay our hands

on and look at the specificity of protein synthesis to see which amino acids were going into protein. We tried. I got hold of some tobacco mosaic virus RNA, and it was superb as a template, compared to the ribosomal RNA preparations which had only a small contaminant of messenger RNA; 1 or 2 percent of that RNA which was messenger RNA.

Synthetic polynucleotides were the next obvious step. It didn't take any real genius to think of these things. They were the most obvious things you could imagine. I rounded up some poly-A, ***polyuridylic acid (****poly-U****) and polyadenylic acid***, some poly-C as well, from people at the NIH.

We missed the template activity of poly-A at first because poly-A is a template for the synthesis of polylysine, a very basic protein, which is not precipitated by trichloracetic acid. What we did at the end of each experiment was to precipitate the protein with trichloracetic acid and wash the precipitate and then count the radioactivity in the precipitate. Polylysine was soluble. The product was soluble in trichloracetic acid so we didn't see any amino acid incorporation. Severo Ochoa was the one who first found this.[4] In Ochoa's lab they used a different protein precipitation method and detected the incorporation of lysine into polylysine, the synthesis of polylysine.

RH: What about equipment? And space? How did you go about getting what you needed?

MN: There wasn't that much equipment at that time. You don't need that much. You need an ultracentrifuge, some other kinds of centrifuges, some counters, and a scintillation counter. That is about it and then a few little ***electrophoresis*** apparatuses. We got one electrophoresis apparatus that was a tremendous monster. For many years it was in an exhibit case over in Building 10.

About this time, I devised the Millipore filtration assay for protein precipitates. One night I decided to do an experiment to compare the amino acid incorporation into protein by the usual Zamecnik-Siekevitz protein precipitation method and a new method that I thought of that would use trichloracetic acid to precipitate protein.[5] My next step was to pour the precipitate on Millipore filters, which were filters with homogeneous pore size, and then try to wash the precipitate on the filter. It took seconds, not an entire day, to do washing like this, and the results were identical.

I had heard about these Millipore filters. They were new. I had heard that Bruce Ames had used these filters so I got some filters from him. Using this new technique was important because it cut way down on the time that was required for an experiment. It used to take both of us two days to do a single experiment, and now we could each do an experiment every day. We increased our output by more than five-fold. It was a tremendously important technical advance in that it allowed us to accomplish a lot more. I never published the assay as part of a paper, but eventually everybody learned of it and started to use it. I did publish it in *Methods in Enzymology* once, but that was much later. The work depended upon the availability of the materials. You couldn't buy these

materials. You either had to make them yourself or get them through people at the NIH. The NIH is wonderful because there are so many people doing different things that it was possible for us to get some as gifts.

At that time, there were maybe only five or six people in the world who were experts in nucleic acid biochemistry. Leon Heppel was one of them, and he was the real McCoy. He had a postdoctoral fellow, Maxine Singer. She was an expert on polynucleotide phosphorylase, the enzyme that catalyzes the synthesis of these randomly ordered polynucleotides.[6] I knew of Heppel and Singer's work. I knew most of the biochemists here at the NIH and what they were doing. ***These*** were only two people here who were experts working in nucleic acids at the time, so, of course, I would know them. Both of them played important roles.

Heppel was such an interesting person, a marvelous person. I can't explain it, but he worked in the lab all the time. He was in the lab from morning until night. He had to be the first person in the laboratory and usually was the last person to leave. He was a small, thin, wiry bird-like type of person, and he sat down infrequently.

My problem was that he worked all the time, and I didn't want to interrupt him when he was working. I didn't want to wear out my welcome. When could I get a chance to talk to him? He was an enormous resource for me. He knew everything that he had done, and he had kept most of his experimental products. They were byproducts of previous experiments that he had done, and he was extremely organized. He had a number of deep

freezers, and he would store these samples in these freezers. He would say something like, “Yes. I did an experiment that made some of this product three years ago.” He would be able to find it, and he would give it to me. His lab was very important to me. I got poly-A, poly-C, poly-U, and some others from him.

One time, when we were trying to devise methods for making a synthetic oligonucleotides—some known sequence to determine the sequence of codons— Heppel suggested a method of making many of the oligonucleotides that was based on a method that he had used. He had one sentence about this in a paper of his of which I was totally unaware. That was to use pancreatic RNAse A to catalyze the synthesis of triplets from doublets as an acceptor and either U or C 2',3' cyclic phosphates in the presence of methanol.

Pancreatic RNAse degrades RNA. It cuts it into smaller pieces. This was using the ability of the enzyme to catalyze transfer of a monomer to the end of a doublet to make a triplet. He had observed this reaction in one of his experiments and had simply put one line in his paper about it. It worked like a charm, and we used it. We had two major methods for synthesizing the triplets, 64 of them, and most of them were brand new compounds that had never been made or isolated before. This was a method that we used, and it was his idea. So he played a tremendously significant role in our work.

Maxine Singer also was an expert in polynucleotide phosphorylase, which we used to catalyze the synthesis of triplets of known sequence. [Philip] Phil Leder, who was my colleague, worked with Maxine.

Poly-U

MN: But back to the poly-U experiment. Poly-U was the first thing that other people had made that really worked.

The idea was to look for a synthetic template for protein synthesis. I had always assumed that there was a code, that the nucleotide sequence in a nucleic acid determines protein synthesis, that is, the amino acid sequence. I don't know how widespread this assumption was. I don't know whether other people felt exactly the same way about it, but I assumed it.

I used synthetic polymers because I thought that they would have template activity. The experiments were designed to detect the specificity of the synthetic polynucleotides in directing the synthesis of proteins. They were set up to find out which amino acids were stimulated to be incorporated into protein.

The whole straightforward, simple idea that I had was to use the synthetic polynucleotides and to look and see if a synthetic polynucleotide would be a template for the synthesis of a protein containing only one or a few amino acids.

It was clear that I needed a more sensitive assay. I wanted to use radioactive amino acids to examine the incorporation of the amino acid and just repeat the kinds of experiments that I had been doing. I thought that I would have sufficient sensitivity to find the right conditions, the optimum conditions, for protein synthesis.

Heinrich Matthaei[7]

MN: Heinrich Matthaei was the first postdoctoral fellow whom I worked with, the first real person actually whom I worked with because I didn't have a technician. I had worked only by myself. He came after I had been doing this work for about a year and a half.

In 1960, Heinrich Matthaei came to the NIH. He came to the NIH (campus), walked into a lab in the first building that he saw, the lab of Roy Vagelos and asked, “Who at the NIH was working on protein synthesis?” Roy called me up and said, "There's somebody here who's interested in protein synthesis. Should I send him over to your lab?" So I said, "By all means." Heinrich thought that with such a large institution there must be lots of people who were working on protein synthesis. I was the only person at the NIH who was doing cell-free protein synthesis and so Roy Vagelos told Heinrich about me. So Heinrich came. He had a NATO fellowship. So it was simply a matter of transferring the fellowship to me.

Basically, what I was doing was having Heinrich repeat the various experiments that I had done before he had come to the NIH, but with a more sensitive assay. When Heinrich

came, I had invested about a year and a half in research, and I had set up all the systems. I had systematically investigated different ways of modifying the components, trying to improve the system, the concentration of every component that went in—pH, all the variables of the reaction. I thought I was getting an increase in penicillinase activity. I finally realized I needed a more sensitive assay than trying to get an increase in enzyme activity, penicillinase activity.

We needed to study radioactive amino acid incorporation into protein. This is what I proposed for Heinrich to do, to repeat some of the key experiments that I had done when I would vary the components' reaction mixtures to optimize cell-free protein synthesis. Since I had done every one of these experiments, I simply rewrote the protocols for the use of C14 amino acid rather than an unlabeled amino acid for him to do, so this was systematic variation of the conditions that were needed for cell-free protein synthesis.

RH: What particular expertise, different from what you had, did Matthaei bring to the work in general that you did over the year and a half or so that he was at the NIH?

MN: When he came and since I had already done it, I knew exactly what experiments really had to be done; so I wrote down all the protocols for these experiments. I did something I shouldn't have done basically because Heinrich was a postdoctoral fellow. But I thought it would be the most efficient and the quickest way, and, in fact, it was a very quick, fast way. Within a very, very short time we really had a beautiful system.

To ***determine whether poly-U could direct protein synthesis,*** we needed 20 different kinds of solutions, each containing nineteen of the amino acids and lacking a different amino acid. Solutions had to be made up with nineteen unlabeled amino acids, cold amino acids. Then, each ***solution*** was supplemented with one different radioactive amino acid that then would be used to check for the assay. We had a separate solution for each kind of radioactive amino acid so that we could look for the incorporation of that amino acid into protein, dependent upon the addition of a synthetic polynucleotide. That was the way the experiments were designed. We were looking for the activity of the polynucleotide as messenger RNA.

I wrote protocols for Heinrich, who began to do these experiments, and the whole experiments were designed to look for and test the incorporation of each of the 20 amino acids separately in separate tubes. Solutions had to be made up with 19 unlabeled amino acids, cold amino acids, and one radioactive amino acid, a different radioactive amino acid in each solution that then would be used to check.

He was trained as a plant physiologist. One thing he insisted on, right at the beginning, was that he would make all the 20 radioactive amino acids. He had experience. He had done this before in Germany. He started from C^{14} bicarbonate and used algae that incorporated the CO_2 and synthesized radioactive amino acid into protein, precipitated a protein from the algae, hydrolyzed the amino acids, then separated them, fractionated them, and obtained his preparations —20 different radioactive amino acid preparations.

I really didn't want to do that because I would have preferred to buy them. It was quite expensive to purchase them, probably $200 or $300 dollars a piece and for 20 different amino acids; but he said that he had done it before and insisted on doing it. I think he probably felt that they were purer than you could get commercially also. He insisted actually on doing it this way because he had had experience in doing it; so they were really well made. So initially, at least, we used his radioactive amino acids. It took him a little while to make them. But he was excellent in the lab.

I couldn't have done it without Heinrich. I couldn't have done it alone. The labor would be too much for me. Heinrich was meticulous in the laboratory. He was an excellent investigator and worked very hard. These protein synthesis experiments —the way we were doing the work —took two days; and he would work during the day, and I would take over at night, for example. We worked in shifts part of the time.

The fact is that before he came, I had done almost every one of the experiments leading up to this by looking at the conditions that were needed for protein synthesis with a crude assay enzyme activity.

What we needed to do was to repeat all of those experiments with a more sensitive radioactive amino acid incorporation assay. Since I had already done that and because I had done these things and knew exactly what to do, it was very easy for me to do it. He had no prior experience in protein synthesis. It wouldn't take any time at all. It had taken me a lot of time to do them initially. I knew exactly which experiments had to be done,

and I wrote the protocols for the first year that he was here.

I wrote every protocol for him; so I just got in the habit of writing protocols for him. It didn't take much to write a protocol because he knew the systems, but that was a mistake for me to do that. You don't write protocols for a Ph.D., for a postdoctoral fellow. Each person should write his own protocols.

After we had done this for about a year, he exploded one day. It was the first time I had ever seen him angry. Apparently he had been angry for a considerable time for doing this, and I should have understood and had stopped that. I immediately stopped writing protocols. He told me that he didn't want me to write the protocols. He was perfectly competent to write the protocols himself. By that time he was perfectly competent to write the protocols because we had been working together for a year.

When he told me that he wanted me to stop, I immediately stopped doing that, and that certainly removed the source of friction. I think, at the beginning, it was justified to write protocols for the work because I had done these things and I knew how to do them, and it saved him time for me to do this. But I should have stopped doing it earlier than I did. Afterward he wrote his own protocols, and I thought things were going extremely well.

Before I left for Berkeley, I left Heinrich a series of protocols of experiments to do while I was gone. Basically, what I was doing was repeating the various, the same experiments that I had done before Heinrich had come here, but with a more sensitive assay.

Now all of this was simple and straightforward. I assumed that if there was going to be a template for protein synthesis, that it would be present on ribosomes because ribosomes were required for protein synthesis at the time. Ribosomes have plenty of RNA, so I thought that by making ribosomal RNA preparations that a small part of that preparation would be template RNA. We tested this and, sure enough, it stimulated protein synthesis very slightly.

Our major problem was that we had a high background of incorporation of amino acids in the cell-free system. I switched to *E. coli* because there were background of counts from penicillinase synthesis. I found that by treating the extracts with DNAase first, we could reduce the background.

The very first experiment with the ribosomal RNA worked, but the counts were only about 30 counts above background. I remember coming back ***to the lab*** from supper to look at the counts from this first experiment. Everything was in duplicate. We had all the controls there, and it worked. It was 30 counts above background, and I jumped for joy. I let out a real whoop and I said, “We've done it! We've got it.” That was the first indication that we had that RNA was a template for protein synthesis. Basically, that was the discovery of messenger RNA, the cell-free assay for messenger RNA.

I am thinking of an experiment that I did to lower the background, which worked like a charm. There was a big background of cell-free protein synthesis, background of cell-free protein synthesis. Experimentally I had to reduce that endogenous amino acid

incorporation to increase the sensitivity of the assay. I found that this could be done by treating the extract with DNAse, incubating with DNAse to destroy the DNA and to allow endogenous messenger RNA to direct the synthesis of protein: and then reactions would stop. Then I would add new messenger RNA and the reactions would come up from almost zero. It was a much more sensitive way of assaying. That was what at first I thought that this was. I am not so sure that this is now, but I certainly used "Synthesize messenger RNA. Add back to ribosomes."

We thought synthetic RNA was going to be an active control, a template. We thought it was going to be messenger RNA. That was what we looked for. We looked for the coding properties, the ability of these synthetic polynucleotides to direct cell-free protein synthesis. It was the most obvious thing in the world. ***And*** we found that poly-U directed the synthesis of polyphenylalanine.

From then on, everything was simply to try to optimize the system, to find out what components were required and to find out what the optimum concentrations of the components were. I got hold of as many different RNA preparations as I could.

Perola got some tobacco mosaic virus for me from George Rushizky at the National Cancer Institute, whom she knew. This was a viral RNA. When I added that viral RNA to the reaction mixtures, instead of getting a small number of counts, I got thousands of counts going into protein. That was really hot as a template, more active than ribosomal

RNA. We fractionated the ribosomal RNA and showed that only a fraction was actively active as a template, as I had expected, like 1 or 2 percent of the total.

Knowing that we got messenger RNA there, it was obvious what we had to do. ***We had to do*** incremental explorations to see if we could improve the activity of the system. Since synthetic RNA had been made and prepared, it was absolutely obvious to test it to see if it acted as templates for protein synthesis, but there was nothing funny or unusual about it. It was standard, we were just looking at the specificity of the RNA.

So the ***first poly-U preparation*** I got from somebody at the NIH was from [Daniel] Dan Bradley. He died years ago. He was a physical biochemist. I think he was in either the mental health institute [NIMH] or the neurology institute [NINDS], and he was studying the properties of the poly-U. Actually this was shortly after we found that the tobacco mosaic virus was a terrific template, much more active than ribosomal RNA.

Victor Ginsburg, who was a biochemist in the next room, also in Gordon Tomkin's section, had gotten his Ph.D. from Berkeley, and he knew Heinz Fraenkel-Conrat. [8] Fraenkel-Conrat was a professor in the department of biochemistry at Berkeley and was one of the world's authorities on tobacco mosaic virus RNA. Fraenkel-Conrat had mutants of tobacco mosaic virus. I thought that what the viral RNA was doing was acting as a template for the synthesis of viral coat protein. That was the simplest explanation because it was so active as a template, fantastically active. I thought that this was so important and so exciting that to do this, ***but*** to report this, you really had to be able to

prove it. You had to do it right. I thought that if I collaborated with Fraenkel-Conrat, who had the mutants, and if we were really making viral coat protein, we should characterize the product and show that with wild-type RNA you would get one kind of protein and with a mutant of the coat protein you would get an amino acid replacement position in that protein.

Vic called up Fraenkel-Conrat and introduced me to him over the telephone. I told him what we had found. I thought that our result was extraordinarily exciting. So did he, obviously. He invited me to come out to Berkeley to work in his lab to prove it.
The objective was to use tobacco mosaic virus RNA that Fraenkel-Conrat had. He also had some mutants of tobacco mosaic virus that had mutant in the gene for the coat protein, the viral protein. I set out to prove that we were synthesizing a protein directed by nucleic acid that we would use both the wild type RNA and the mutant RNA. I thought that we should see an amino acid difference in the appropriate amino acid-peptide sequence by comparing the mutant protein to the wild type protein. That would pin it down beautifully. That was the objective.

It was a three-way collaboration. I did the work on the protein synthesis, and Fraenkel-Conrat prepared the RNAse, the mutant to the wild-type RNA. Akira Tsugita, a post-doctoral fellow on sabbatical from Japan, was an expert on amino acid sequence analysis. He did the purification of the radioactive peptides after the tobacco mosaic virus coat protein was cleaved. He would characterize the protein product and show that there was a particular amino acid replacement in a particular mutation. He had considerable expertise

and was recognized for this expertise in characterizing the amino acid sequences of proteins and peptides, fractionating peptides, and in determining protein structure. When we thought we were directing the synthesis of tobacco mosaic virus, he was in charge of the characterization of the protein that was synthesized in the reaction mixtures. Then peptides were separated, and we were looking for a change in the incorporation of a particular amino acid, phenylalanine, in one of the peptides.

In fact, to tell you the truth, the way it worked was different. Although the RNA was extremely active as a template for protein synthesis, we were not synthesizing the tobacco mosaic virus coat protein as we thought we were. This was because the opposite strand, the complementary strand of the virus, was the template for coat protein synthesis. In other words, the viral RNA had to be copied into its complement, which served as the real messenger RNA. Although we were definitely synthesizing a particular protein that was encoded by the viral RNA, it was the wrong strand, so biologically it was not the protein that was related to the viral replication or synthesis. We happened to be wrong on that one.

We published a paper in *Proceedings of the National Academy of Sciences* about our findings when we thought that it was the viral coat protein. But, Tsugita's analysis was wrong. What Tsugita did was to simply isolate the fractions coming off of the column. He threw away and did not analyze in between the fractions of the peptides. The peptides would come off the column one after another and be collected, and he did not analyze the material in between the peptides. He knew what the peptide fractions were supposed to

look like. That was an absolute mistake, and it gave the wrong results. So we were wrong when we said it was the coat protein.

His error taught me a real lesson —that you have to be responsible for every part of the paper. Even though the three of us were doing separate parts of the paper and we were all responsible for what the paper said.

When we found out about it, Fraenkel-Conrat and Tsugita redid the work. I furnished the radioactive proteins that were synthesized with the thing, and we published a retraction. Fraenkel-Conrat told me he had planned to publish the retraction by himself with Tsugita because he didn't think that I was involved in that. But I refused to do that. I thought that I was just as responsible as they were, even though I had nothing to do with that part of the work. It was my responsibility to share the blame, for being wrong in the paper that we had originally published on it, so the three of us published it together.

I admired Fraenkel-Conrat. I first met him when I went to his laboratory and worked with him, and I admired and liked him. He was a tough guy with an aggressive and sharp tongue and smart. I liked him because he worked in the lab, and he insisted on doing his own experiments, even though his students did experiments. But every day he did an experiment, and he arranged his life so it was possible for him to do this. That I admired. I thought then and think today that he was an excellent scientist, first rate. It was the best laboratory I have ever worked in. I liked him personally. He was considerably older than

I, much more experienced, and tough, rough, but the real McCoy. I think very highly of Fraenkel-Conrat.

While I was working out in Berkeley in Fraenkel-Conrat's laboratory, Heinrich called me. He told me that he had done the poly-U experiment and that it was fantastic, that it worked, that it stimulated the incorporation of only phenylalanine into protein: we were synthesizing polyphenylalanine. It was clear that a sequence of U's coded for phenylalanine, this was the first codon that was deciphered, and it was clear that this was an approach that could be used to gain a tremendous amount of information about the genetic code. It told what bases were involved in nucleotides, but it didn't tell the sequences of the nucleotides.

I want to emphasize again, I couldn't have done it without Heinrich. We used the 20 different radioactive amino acids that he separated for the experiments. He insisted on doing it this way because he had had experience in doing it, so they were really well made. He was a very excellent experimentalist.

RH: Were you surprised at the outcome of the poly-U experiment after all the others?

MN: Not surprised. Overjoyed is the way to say it. But not surprised at all because it was RNA, and that was the objective—to look for the activity.

RH: There is a story that I want you to comment on that was told to me by one of your colleagues. Apparently, and this is third-hand, Heinrich told Gordon Tomkins what happened with the poly-U experiment and said he was very disappointed. Tomkins said that obviously Matthaei didn't understand the importance of what had happened in the experiment.

MN: That I have never heard. Before I left for Berkeley, I wrote protocols for all the experiments that Heinrich was to do while I was at Berkeley because I had been doing this all along with him. Included in this batch of protocols was the experiment to test the specificity of poly-U. It was done just in the way I said it was with 20 different solutions of radioactive amino acids. I think it would be impossible for Heinrich not to understand what the objective was. He understood perfectly well what the objective was. I have never heard that story before. He did call me when I was in Berkeley for three or four weeks. I was working hard there and making radioactive proteins for Fraenkel-Conrat and Tsugita.

I am certain that Heinrich had an absolutely clear idea because he had to make all the reagents for this, all those solutions. There was only one reason to make these solutions: to look for the specific incorporation of a kind of amino acid into protein, directed by a template with only one kind of base. I think the story is highly unlikely to be true.

One thing I can say for certain: when Heinrich told me on the phone that the poly-U worked, he knew exactly how exciting it was. He was very excited about it, and so was I.

It was a terrific experiment and a terrific finding, and it just opened up a world of things for us to do. It was tremendous. Heinrich is highly intelligent and obviously understood everything, so I don't believe it. It was obvious that I had to go back immediately. He was very excited about it, and I was very excited. It was fantastic.

I had to leave Berkeley and come back home. Fraenkel-Conrat invited me to give a talk on the tobacco mosaic virus work that I was doing there before I left, so I did give a talk. I didn't mention anything about the poly-U, but I immediately went back to Bethesda because it was so exciting.

We published the first paper on the effect of ribosomal RNA, of a contaminant in ribosomal RNA, in *Biochemical Research Communications*. That was the first paper I had published in two years. Basically, the discovery of messenger RNA was in that paper although we couldn't say that it was messenger RNA; we just said it was RNA that stimulated the incorporation of amino acids into protein.

The footnotes that appear below will be placed in a separate digital file for linkage to this file.

[1] Bruce Ames (1928-) earned his Ph.D. at the California Institute of Technology. He served as a fellow and then biochemist from 1953 to 1967 in the National Institute of Arthritis and Metabolic Diseases.
[2] Paul Charles Zamecnik (1912-) helped open new areas of research in genetics and pharmaceuticals. He described how cells turned the genetic code of DNA into cell building proteins. A graduate of Dartmouth with an M.D. from Harvard Medical School, he conducted research and served as director and as a professor of oncologic medicine at the Huntington Laboratory at Harvard. After retiring from Harvard in 1979, he conducted research at the Worcester Foundation and the Hybridon Company. He received the Lasker Award in 1996 and the National Medal of Science in 1991.
[3] François Jacob, Jacques Lucien Monod and Andre Michael Lwoff received the 1965 Nobel Prize for Physiology or Medicine for their explanation of gene expression.
[4] Severo Ochoa (1905-1993) received his M.D. from the University of Madrid in 1929. He came to the New York University School of Medicine in 1942 and served as research associate, professor, and later chair of the Department of Biochemistry. Ochoa shared the 1959 Nobel Prize in Physiology or Medicine with Arthur Kornberg.
[5] Philip Siekevitz earned an undergraduate degree in 1942 from the Philadelphia College of Pharmacy and a Ph.D. in biochemistry from the University of California, Berkeley, in 1948. He and his colleagues at Berkeley were among the first scientists to use radioactive amino acids in protein synthesis research. In 1949 he became one of the first to receive an NIH postdoctoral fellowship at Harvard's Massachusetts General Hospital. He later conducted research at the University of Wisconsin, Madison, the Rockefeller Institute, and Yale University.
[6] Maxine Singer (1931-) received her Ph.D. in biochemistry from Yale University in 1957. She was a postdoctoral fellow of the Public Health Service at the National Institute of Arthritis and Metabolic Diseases from 1956 to 1958, and a research chemist in the institute's Section on Enzymes and Cellular Biochemistry from 1958 to 1974. She later became president of the Carnegie Institution of Washington.
[7] Heinrich Matthaei (1929-) received a degree in plant physiology from the University of Bonn in 1956. He came to Cornell University to work on a one -year postdoctoral NATO fellowship in the laboratory of Frederick Steward, a botanist, to conduct research on cell-free protein synthesis. Marshall Nirenberg wrote on September 30, 1960, in his diary, Binder IX A, of work to do "When Heinrich comes."
[8] Victor Ginsburg (1930-) received his Ph.D. in biochemistry at the University of California, Berkeley, in 1955. In 1956 he became chief of the Biochemistry Section in the National Institute of Arthritis and Metabolic Diseases. Heinz Fraenkel-Conrat (1910-1999) received his M.D. from the University of Breslau in 1933 and his Ph.D. in biochemistry from the University of Edinburgh in 1936.