Translating the Code of Life – the "Race"

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:

From NLM "Profiles in Science:"

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

Despite their initial success, Nirenberg and Matthaei's newfound celebrity produced some unintended consequences. While the poly-U experiment was proof that they had in effect "cracked" the genetic code, many scientists–especially the 1959 Nobel Laureate Severo Ochoa–were eager to take it to the next level. Once it was understood that UUU was the RNA "code word" for phenylalanine, scientists set out to discover the unique code words for the twenty major amino acids. With this knowledge, scientists theorized, one would know not only how RNA translates messages from DNA to build proteins, but one would be able to read the entire genetic code of living organisms. As Jerard Hurwitz and J. J. Furth asserted in *Scientific American* in 1962, understanding the genetic code would explain how "the dream of the gene [becomes] the reality of the protein." Many believed, as the *New York Times* suggested in 1961, that "the chemical code of inheritance, which determines the form and function of every living thing and thereby provides the basis for genetics, will be cracked before the year is out."

Suddenly, the young NIH scientists found themselves in a research race with some of the world's most famous (and well-funded) molecular geneticists. Over the course of the next five years, Nirenberg worked steadily with a team of about twenty postdoctoral researchers and laboratory technicians, including Norma Heaton, who has remained a member of Nirenberg's team at the National Heart, Blood, and Lung Institute for forty years. Using the three-letter poly-U experiment as a kind of paradigm, the Nirenberg team extended its experiments with synthetic RNA even further. During this period, they discovered that AAA (three adenosines) was the code word for the amino acid lysine, and CCC (three cytosines) was the code word for proline. GGG (three guanines) turned out not to work as a messenger at all. They also discovered that by replacing one or two units of a triplet with other nucleotides, they could direct the production of other amino acids. They found, for example, that a synthetic RNA composed of one unit of guanine (G) added to two units of uracil (UU) directed that valine be added to a developing amino acid chain. In Nirenberg's shorthand method, the code word for valine was GUU.

In the late 1950s, the biochemist Sydney Brenner coined the term "codon" to describe the fundamental units engaged in protein synthesis, even though the units had yet to be fully determined. Francis Crick popularized the term in 1959. After 1962, Nirenberg began to use "codon" to characterize the three-letter RNA code words. With one of each of the four nucleotides occupying a place in a three-letter codon arrangement, Nirenberg quickly deduced that there were 64 possible combinations (4 x 4 x 4) of three-letter codons. Viewers can see some of Nirenberg's original charts that show the process by which he kept track of the various combinations of nucleotides in the RNA codons in the Documents section. In 1964 and 1965, Nirenberg's postdoctoral researcher, Philip Leder, developed a

sophisticated filtration machine that helped the team determine the order of the nucleotides in the codons. This development speeded up the process of assigning code words to amino acids. By 1966, Nirenberg announced that he had deciphered the sixty-four RNA codons for all twenty amino acids. This remarkable personal and scientific accomplishment held great significance, not only for Nirenberg but also for the history of modern science. At a 1966 conference, Geoffrey Zubay, a professor of biology at Columbia University, remarked that "Francis [Crick] ... predicted the entire code would be solved in [1961]–and it has taken a few years longer than that and the pace, in spite of [Crick's] prediction, has been miraculously fast. I think it fair to say that Marshall Nirenberg has carried the ball all the way."

In a 1967 talk, Nirenberg characterized messenger RNA as a "robot" whose purpose was to obey the commands of DNA and carry out vital genetic instructions. "Man," Nirenberg observed, "now understands the language of the civilization, has written quite elementary messages in the form that robots understand, and via such texts has communicated directly with the robot. The robots read and faithfully carry out the instructions." For others, however, the idea of a code that controls our genetic make-up depended on less futuristic metaphors. As the 1958 Nobel Laureate George Beadle and wife Muriel Beadle wrote in 1966, "the deciphering of the DNA code has revealed our possession of a language much older than hieroglyphics, a language as old as life itself, a language that is the most living language of all–even if its letters are invisible and its words are buried deep in the cells of our bodies."

1995-1996 Harris Interviews

Ruth Harris (RH): Now we will talk about the race to decipher the genetic code which took place from 1961 to 1964. Can you tell me about that time?

Marshall Nirenberg (MN): Hans Stetten called it "the finest hour of the NIH," and I think that's a pretty apt description because people like Bob Martin stopped what he was doing — his research— and came to help synthesize these polynucleotides and to make the things; and that was crucial, absolutely crucial, at that time.

Well, the race first started soon after I had presented our work in Moscow. We picked up the *New York Times* and saw, I think it was on the front page, an article about Severo Ochoa. He had called a press conference. I think that that was what started the race. The way that he said it was, basically, that he and his colleagues could decipher the genetic code using his technique. In fact, he was the most experienced person in the world in work related to this and had many of the polynucleotides already made.

Then, Hans Stetten [the Scientific Director of the institute] called a press conference in which he said that I had described in Moscow a technique that could be used to decipher the code. That was the start of it. So deciphering the code took about five years, five of the most intense years I have ever spent.

Severo Ochoa

MN: I heard rumors that people in Ochoa's lab had used other polynucleotides, randomly ordered polynucleotides, and had gotten other amino acids into protein, but I discounted those rumors. I didn't think that they were true. I called Ochoa because I wanted to meet him, and I thought that maybe he wouldn't be so competitive if we were to meet. He invited me to come down to his department and have tea, which I did, and he introduced me to everybody in the department.

Ochoa was a gracious, older biochemist, one of the most famous biochemists in the world. He was president of the International Union of Biochemistry and looked like a

Spanish grand nobleman. He spoke with a slight Spanish accent and had a very wonderful manner. But he was an absolutely ruthless competitor, and people didn't like that basically. I understand the thing from his point of view, though. I have heard from postdoctoral fellows who were in his lab that at tea every day—they had a tea every day—he would ask people what the latest results were in their experiments because he was interested in their experiments. They didn't like to tell him what the latest results were because they wanted to work it out themselves more or less and then tell him.

Somebody, possibly one of the postdoctoral fellows working in Ochoa's laboratory, told me that once in a conversation with Ochoa, he inadvertently spilled the beans about a month's work that he had gotten ahead of telling Ochoa, and that now he was totally caught up, so Ochoa would suggest experiments. But, basically that was not fair to Ochoa because he had a genuine interest in the work and if people came to his lab to collaborate on projects, of course he would be interested in their work. Ochoa was a fierce competitor and a magnificent biochemist. He was the father of enzymology in the United States.

Ochoa had a large lab, and had experience in nucleic acids. I had neither of these. This was the first independent problem that I had worked on. I had no experience in nucleic acids, only the experience in protein synthesis that I had gained in the previous two years when I was working on this, and I was working with only one postdoctoral fellow. It really was a race, and Ochoa was such a fierce competitor that I think that it turned off people. I went down to visit him. I thought that if we met one another, it would be better.

Given the choice, I would much prefer to collaborate with people, not to compete, although I found that I liked to compete too. I never realized this until I was actually forced into this situation with Ochoa. It kind of scared me because I always thought of myself as being much more civilized than that. It is true that I always have been helpful to people, but I found, to my real surprise, that I enjoyed competition. It was a stimulus. I didn't really care if I lost, and I didn't really care if I won, but it was a game to play, and the point of the game was to try. Basically, I was interested in the problem and the competition. Whether I won or lost due to the competition was of secondary interest. But, I did like the competing.

I have to be honest and say it, although I am a little repelled by that discovery about myself. But that is the truth of the matter. My own inclination is to collaborate and cooperate wherever possible as it is easier to make all the changes and adjustments that need to be made. I never let competition worry me. I just tried to do the best that I could. I was doing it for the work, for the fun of the work, basically. But there was no way to split up the work, and it didn't do a bit of good. He was just as competitive as he could possibly be.

The "race"

MN: (It) was a stimulus to get things done, and I got things done. I never missed a deadline for getting a paper out, and it helped me. Basically, if you are doing the work

yourself as I was, or maybe with a very small group of people, I think that I had an advantage compared to Ochoa, who had a big lab, who was the most experienced person in the world with synthetic polynucleotides. He had a whole library of synthetic polynucleotides that he had made. He had won the Nobel Prize for doing this work. But we went toe to toe for a number of years, paper after paper, reporting the base compositions of codons.

One time coming back from a symposium where I had spoken, I found I had been given the seat next to Ochoa, who had also spoken at that meeting. So I sat down. He was reading *Anna Karenina*, a Tolstoy novel, and I was reading the latest *Nature*. We talked for a while and eventually went back to our books. But years later after the code I got to know him because we were both members of the Vatican Scientific Organization. At a meeting at the Vatican we stayed in the same hotel, and I met his wife. Perola really liked his wife very much. They went shopping in Rome together. He was very gracious, and he definitely had two totally different sides. But his fierce competitiveness kind of turned people away from him ultimately, I think.

RH: So what was the actual work involved? Could you please describe how you deciphered the genetic code?

MN: I guess you could say that the code was deciphered in two phases.

Phase 1 - determining the base composition of codons

MN: The first phase was to determine the base composition of codons. We synthesized every possible combination of the four bases. Not only poly-U—only RNA with one base, but RNA with two bases, three bases, four bases—the three bases and the two bases in all possible combinations. We used these as messenger RNA to direct the cell-free incorporation of radioactive amino acids into protein.

If you take poly-AC, for example, by varying the proportions of the percent of A and C in the RNA, you could calculate the expected abundance of codons with one A and two C's, or with two A's and one C. We did this, and we synthesized many, many polynucleotides.

Bob Martin also worked on this during the early phase of the translation. Then we analyzed the RNA preparations for their base composition, determined the base composition, of each one, and made the calculations as to the expected probability of getting different codons. When we plotted the data, it was quite clear that we could easily distinguish between codons that had, for example, two A's and one C, or one A and two C's. We did this for each combination of bases, of nucleotide residues.

It was also clear and the work really strongly suggested that the code was a triplet code just because it fit the expected frequencies of codons so beautifully.

One time, during the first phase of deciphering the code, I was absolutely horrified to find that our liquid nitrogen freezer had disappeared with all of our ribosomes and enzyme preps and everything else. At that time we stored the samples immersed in liquid nitrogen. They were portable. Yes, and every week somebody came and took our empty reservoir someplace, filled it with liquid nitrogen, and brought it back to us. The person —I guess it was a new person —mistook the freezer, the thing that we had all our samples in, for the reservoir. He took it all the way across the District of Columbia to the plant to refill it with liquid nitrogen. It had in it all of our precious materials that we had spent months in preparing. Of course, when they discovered that there were samples in it, they immediately brought them back, jouncing on that truck. Nothing was hurt that I know of, but it was a tremendous shock to find out that all these samples disappeared.

There is an interesting story that I can tell you here. We found an extra amino acid, methionine that was being incorporated by poly-U/G, which was unexpected, because we knew how many triplets you could get out of poly-U/G. We had assigned the triplets to each one of all the amino acids. So the first thing that came to mind was, "Well, we've got a contaminated amino acid."

There are two amino acids here that are going in rather than one. We were just looking at radioactivity. Heinrich Matthaei spent about a month purifying every one of the amino acids that would be incorporated into protein to see if there was contamination. There was no contamination. We thought it might be nucleotide contamination when we synthesized the polynucleotides. He spent a lot of time looking at that. We came up

without any explanation for it. We simply reported the fact and didn't say anything about it because we had no explanation for it.

Phil Leder, Maxine Singer, and Marianne Grunberg-Manago solved the problem with polynucleotide phosphorylase, and Mert Bernfield solved the problem for the synthesis with RNase A. So we had our methods. It was just a matter of doing it and of getting it done fast, as fast as we could, and as well as we could.

Philip Leder and the First Triplet Paper

MN: Phil Leder, a postdoc, was supposed to transfer, to leave fairly soon, in a few months. He was going to work in another laboratory to gain some additional type of experience. I told Phil that we had a way of determining nucleotide sequences, and I showed him the results. So instead of leaving the lab, he decided to stay to work on this problem. I went to him and told him that we had a technique. I should say that we had tried many different approaches to try to decipher the sequences. We tried to synthesize chemically oligonucleotides of known sequence, and we tried a number of different approaches; but this was the real break in the problem because this approach was so simple, so easy to do, and it would clearly work. The only problem was that there were 64 triplets, kinds of triplets; and most of them were new compounds that had never been isolated or characterized or reported previously. So the main job then was: how do we synthesize, how do we make, triplets of known sequence that we could use in this technique?

Phil Leder found in some journal an advertisement for a half a gram of each of the 16 doublets, the 16 possible doublets. You couldn't buy them. They weren't commercially available anyway. All of a sudden this advertisement appeared in a European journal that somebody who was working in the field had synthesized or had isolated a half a gram of each of the 16 kinds of doublets and was willing to sell them. So I went to Bob Berliner, a wonderful administrator who was our boss here at the time, and told him that we had this opportunity to buy the starting materials and that we could add a single nucleotide to a known doublet to make triplets using polynucleotide phosphorylase. I asked if we could buy them, and he said, "Go ahead and buy them." At that time the cost of those things was a fortune, although by our standards today I think it was very little. It was —I don't know —$1,500 dollars for each one; so we ordered the whole set of 16.

When they arrived, there were only 15 vials, and I sent back a query, "What happened to the sixteenth doublet?" Well, Customs saw 16 vials, each with a little bit of white powder in it, and so they took one of them. They didn't know that it was a precious material, really precious material. It turned out that the American customs agents had taken the entire contents of one vial and had tested it for drugs. They had just tossed it out. They had destroyed it. It was amazing to me. So we only got 15, but we used those as substrates.

Phil Leder went to work with Maxine and Marianne Grunberg-Manago, a visitor in the lab; and the three of them developed a method for using polynucleotide synthesis to

catalyze the synthesis of triplets and higher homologues by adding one base or two bases or three bases. Then we had to purify the oligonucleotides that were synthesized and separate them one from another.

Purifying the Oligonucleotides

MN: I remember Tom Caskey at the time synthesized an enormous quantity of poly-A. It was probably the biggest supply in the world of poly-A, which we wanted to use to treat with nuclease to get oligos, from which to purify oligos. He asked me, “Should we [maybe] do some exploratories to see what conditions to use?” I was in a hurry because we wanted them as fast as we could possibly get them. I said, "Naw, just let's use one condition and hope for the best," and it destroyed everything. We lost the whole batch. We had to start from scratch to make more. That was a very bad piece of advice I gave Tom.

W. Anfinson French was one of the prime movers in purification of the oligos. We had a little factory going. In connection with this once when I went to see Leon Heppel, Mert Bernfield told me that he had found a method that might be useful to us for synthesizing triplets which ended in U or C. That is, he used a nuclease, RNase-A, which destroys oligonucleotides or polynucleotides; it cuts them in pieces. He had found that if you use a 2', 3' cyclic nucleotide phosphate, U or C phosphate, in the presence of relatively high levels of methanol, the enzyme would catalyze the transfer of the 2', 3'. It would cleave the 2', 3’ phosphate and would catalyze the addition of that base to a primer. So we

thought we could use doublets there and add U or C to the end to make triplets. This whole method was a single sentence in one of his papers that I would never have seen. He suggested the method, and it worked like a charm.

Mert Bernfield used it to synthesize almost half of the 64 oligonucleotides. In doing it, Mert became tremendously interested in the mechanism of action of RNase-A. The RNase-A had been obtained in pure crystalline form in quantity at Armour, and it had been used to study the conformation of proteins and something about catalytic activity of proteins. This mechanism was totally different from the deciphering the code, and Mert wanted to stop making these oligos and to pursue his interest on the mechanism of action of RNase-A, which I thought was a very bad idea. Deciphering the code was much, far, far more important than that. So we made a pact that if he made these oligonucleotides, he could then spend the rest of his time in the lab doing his own thing, doing whatever he wanted, and he could publish the papers on the mechanism by himself, which he did. So it worked out well.

Using the two methods of synthesizing the triplets, polynucleotide phosphorylase and the RNase- A method, we managed to synthesize the 64 kinds of triplets, and we purified and isolated them. We then used them to see which aminoacyl tRNA molecules were recognized.

What I did was the binding work, the test, with Norma Heaton and with Teresa Caryk, another technician who worked very hard on this problem. Every day we would test more

oligonucleotides for specificity against 20 different preparations of aminoacyl tRNA. I took care of the testing with Norma and Teresa while French and the boys in the lab made and purified triplets. It was like a little factory to make these things. French really had a lot of responsibility and was in charge of part of the making and purification of the oligos. He played a very important role in obtaining the oligos to decipher.

Towards the end of the deciphering the nucleotide sequences we published a whole series of papers on nucleotide sequences of codons. Phil Leder and I wrote "*RNA Code Words in Protein Synthesis: The Effect of Trinucleotides Upon the Binding of sRNA to Ribosomes*," in 1964.

I reported it at the International Congress of Biochemistry meeting in New York at the time, and it was like a traveling circus. There were symposia, constant symposia, and the same people would speak at these symposia, which actually were held all over the world.

The person who spoke before me at this meeting in New York was Dr. Ochoa, and by accident he had at the end of his talk a slide listing all of his coworkers. There were some 20 coworkers. Then the next talk was my talk, and I started out with my one coworker, who was Phil Leder, and the first slide I presented had Phil Leder's name on it. The contrast between a tremendous number of workers and Phil Leder brought a big laugh from the audience.

Ochoa stopped competing in 1964, when I reported the method of determining the sequences of codons.

Phase 2 – synthesizing the triplets

MN: Ochoa dropped out for the second phase in 1964, and then we were competing against [H. Gobind] Khorana.[1] Khorana came in very late in the game. He chemically synthesized the 64 triplets after we had already deciphered and had synthesized the triplets enzymatically and had identified the codons. He was a competitor at the very end, or past the end of the work. It didn't mean much at the time because we had already done it, or most of it. But it was a race to synthesize those triplets.

Gobind Khorana was the central figure in the chemical synthesis of oligonucleotides at the time. He was the one who devised the techniques and was the leader in the field. I remember meeting Khorana rather early in the work. He had written a textbook, which I really struggled through.

Khorana also made polynucleotides with repeating doublets or triplets and then used them for cell-free protein synthesis, but this certainly didn't give much new information because, again, most of the sequences had been worked out before this. So it was an intense competitive race to be able to do it. I was forced into it. I had to choose either to compete or to get out of the field, and I obviously tried to stay in.

RN: How much contact did you have with Khorana during this period?

MN: Very little. I would see him at meetings, and we'd talk. I followed his work, but we actually had very little contact.

Again, I think that the work was so interesting and so all absorbing that I really wasn't that much concerned about “winning” or “losing.” I was cognizant of the competition, but I never let it worry me. Some things we won, some things we lost. The “race” with Khorana wasn't anywhere nearly as intense or as prolonged as the competition with Ochoa.

Transitions at NIH During the Race

RH: You were initially in the Clinical Center (Building 10).

MN: Yes, Building 10. We didn't have much space at all. We had four rooms. Each room was ten-by-twenty feet. There were about nine of us who could work in this space so we were really crowded. In my room I worked with two technicians, so there were three of us in that room. The technicians were Linda Greenhouse, and then when she left, Norma Heaton, and Teresa Caryk. Initially, when Heinrich and I started, we worked in half a lab, half the rooms. The technicians came later.

I went to Hans Stetten[2], to ask for help because during the early phase of this work after the Moscow conference, I was being swamped. I felt, how could we compete against a big lab? He told me that all positions were filled and all space was filled. You know how things are when there is no extra space in the building, and there are no extra positions. I understood that he would do the best he could but that there was very little that he could do.

He did manage to give me a technician position for Linda Greenhouse. That was a tremendous help. That was about 1962. She was wonderful. She lives in Potomac. She got an MBA. Her husband was a physician, who was doing his residency while she was working as a technician. She had formerly worked in New York at the Rockefeller Institute for Gerry Edelman, who in 1972 won the Nobel Prize for determining the structure of antibodies.

Linda was the first technician Edelman had ever worked with, and he didn't understand how good she was. When I interviewed her, I called him up to get a reference for her and he said, “Yes, she's all right.” She had a mind like a steel trap and she was all business. I would waltz in in the morning and I liked to chat a little bit before getting down to work. She wouldn't want to be interrupted. She would shut me up. She would say, “I'll talk after lunch time.” Or, “I've got to get the experiment started.” She was absolutely terrific.

So, Linda worked on the first phase of deciphering the code — the translation of the work. Then as postdoctoral fellows came into the lab, the lab gradually expanded, but we

only had four rooms, little rooms like this one we are in, and we were really crowded. There were four or five people in the room right next to mine. Our desk was a two-foot place, like a shelf to work on, and it was very tight. It is good to work with fairly limited space, as long as it is not so limited that people get in one another's way, because it is a lively place.

RN: And Heinrich Matthieu left to go back to Cornell?

MN: To go back to Germany. After about two years or a year and a half of working together, Heinrich wanted to be independent, to work independently of me. I thought that things were going fantastically well and that the genetic code problem was such a great problem. It was the most exciting thing that was being done in biochemistry at the time. I thought, "Why perturb such a wonderful situation?" There's more. I wasn't interested in priority. Anything that he wanted was there. He could have taken any part of the thing that he wanted.

I wasn't really that possessive about the research. I viewed it as a shared enterprise, and I hope he viewed it also as a shared enterprise. But he wanted to be independent of me even though I thought, "Why change the situation, break it up?" Since it was so productive and such a good problem, why compete with one another? To me, it made no sense whatsoever, but that's what he wanted to do, and so that's what he did.

RN: ***During this time, you were getting a lot of job offers and you moved from the Arthritis Institute (NIAMDD) where you had worked under Hans Stetten to the Heart Institute (change to the correct name in 1962 NHLBI) where you became chief of the Section of Biomedical Genetics.***

MN: Yes, there was a lot going on. But I made a mistake that I have always regretted with Hans Stetten, who was very good to me, in never telling him that I was getting offers for jobs when I got back from Moscow. I didn't really care about those things. I was interested in getting the work done. I didn't want any distractions whatsoever. I should have told Hans that I was getting all of these offers, but I thought that it wouldn't be fair to do it, to tell you the truth. I thought that somebody who does that was a manipulator, who would use the fact that he was getting offers of jobs from other institutions, to try to increase one's job, to get a higher position, more space, or whatever as a lever that is used to manipulate. I didn't think it was right. I thought that if you did good work, you didn't worry about it. You would get a promotion. So I never talked to Hans about it, and I regret that a lot.

Although Stetten could not offer you a promotion or more space, Sidney Udenfriend arranged for you to get those things by moving to the Heart Institute. What was your relationship with him?[3]

MN: Perola was a lab assistant in his laboratory. Theoretically, he was my boss when I moved to the Heart Institute, but we never really had that kind of a relationship. He had a

very active research group and was very well known in the area of neurochemistry and catecholamine metabolism. He was older than me and certainly far more experienced in the ways of the NIH in terms of the work that I was doing. I was totally independent of him and, as a matter of fact, if I needed equipment or big things, I would go to [Robert] Bob Berliner. You couldn't have had a better person to be an administrator of the intramural Heart Institute research program.

Sid was very fatherly in a sense. He would sometimes give me advice about people, and we would talk every now and then. Perola had a wonderful relationship with him, and she enjoyed working for him. In Brazil they would call her “Doctor,” but in our system her training would be equivalent to a master's degree. He knew exactly the best way of treating her. He relied on her to do the research. He would outline the research in a general way and point her in the right direction, and that was it. Then he would be hands off, and she would solve all the problems. When she had gotten things going, she would report back to him. It was a wonderful way for her to do research because she had a lot of responsibility, which she enjoyed, and she had the fun of solving problems. It was good for him because he didn't have the time to work on the details. It was the best possible relationship. I have never seen a better working relationship ever between two people.

I had gotten an offer of a job from Cal Tech [the California Institute of Technology]. Cal Tech had such a wonderful department of biology that it was the best place in the United States. I thought that if I was ever going to investigate any position, I should look at this

one. The others that came in—I didn't even bother to investigate them. I just thanked the people and said I wasn't interested.

When Perola and I were both going out to visit Cal Tech, she had to tell Udenfriend why she wasn't coming in ***to the lab***. He helped work it out so I could have a lab at the Heart Institute. He made room for us in his laboratory at the end of the corridor, and I think that it was a good interaction because it eventually helped some of the people in his lab switch into protein synthesis. ***Sid*** didn't switch into protein synthesis, but Herbert Weissbach and Herbert Dickerman switched. There was some interaction between the two groups, but mostly we were independent.

If I hadn't gotten what I needed at the NIH, I probably would have taken that Cal Tech position. If I had left, I would have made more money. I would have had more prestige, but I ***wouldn't*** have done what I wanted to do. I have been interested in doing science—and I've done it. You can do it better here than you can almost any other place. To me that is the most important thing. That is what I wanted and that is what I did.

RH: ***You deciphered the code with the help of many colleagues at NIH.*** Can you please elaborate on how you found all these people to help you?

MN: Many people really helped the project. They understood very well what was going on, and this was the first independent project I was working on. I was a neophyte. I didn't have the manpower at all.

With a lot of the people, I knew what they were doing because I had met them. In science you learn what field each person is working in, and then you see papers by them in the literature. It is a community of scientists, and you meet each person and know what to talk to them about, what each is doing, and what each is interested in. Word gets out very rapidly of what people are doing, and so everybody knows what is being done.

There were people who came to me and said that they would like to work on part of the coding. That was how they came. The NIH had some wonderful programs for young M.D.s who had just finished either their internships or their residencies. This was the time when the draft was still in effect, and M.D.s could serve their time equivalent to being drafted by coming to the NIH to do research. Of course, this was a highly desirable thing to do, and so the cream of the crop came to the NIH. It was called the Research Associates Program. Research Associates wanted to come to my laboratory.

Philip Leder was a Research Associate. He had spent a summer doing research at the NIH [with Martha Vaughn]. I think he did neurochemical or pharmaceutical type research before he came to my lab. I don't know if Phil had published anything. He didn't know anything about nucleic acids or protein synthesis. This lab is where he learned initially about those fields. But he did superb work.

Also, there were Clinical Associates who spent half of their time doing clinical work and the other half doing research. It was a wonderful program that the NIH had. Probably the

highest caliber M.Ds. in the United States came to the NIH, and so I was fortunate to work with some wonderful people. They were a highly selective group. There were a tremendous number of applicants, and only the best were selected. They visited the different laboratories, and each institute accepted them separately. They could choose whichever laboratory they would work in if the head of the laboratory had space and would agree to accept them.

I was inundated with letters from postdoctoral fellows who wanted to come to the laboratory, and I would accept maybe one out of 20 applicants for postdoctoral fellowships. Obviously, I looked for experience in appropriate fields, but my own experience showed that people without any experience whatsoever in nucleic acids or protein synthesis would do just fine. It depended on the type of person that they were. They would make the rounds of all the labs, and then we would choose. But most of the postdoctoral fellows came because they had written to me directly. They were getting their Ph.D.s, and they wanted postdoctoral experience. Many of them raised their own funds from different sources. They had a fellowship of one sort or another that would allow them to come here to work for two years. Almost everybody in biochemistry and molecular biology goes to work on a postdoctoral fellowship before starting to work in a university.

I was very choosy about picking people. When I look back on it, it was great to work with some of the best people, some of whom went later on to do wonderful things in science.

There were a number of people who participated in this work. For example, [Oliver W.] Bill Jones came to the lab as a postdoctoral fellow. He was a Research Associate. Bill was terrific. When he was an undergraduate, he had been a football player for the University of Oklahoma, which was a powerhouse at that time. He was a physician and had done some biochemistry before coming to the lab. He was very much involved in this problem of determining the base combinations of codons.

RH: How did you organize the genetic code work with your postdocs? Did you determine the problems that the postdocs would work on?

MN: Sometimes I assigned a particular problem, sometimes I gave a choice. Now I like to give choices because I would prefer to do it like that. Then I might have given some choices also if there were two things of equal importance that had to be done. That is not usually the case. Usually it was one thing that was more important than another. But I picked the problems because they didn't have any experience in the field.

Before he came to my lab, [Thomas] Tom Caskey, who was another Research Associate, had a Ph.D. in enzymology that he had gotten with [James] Jim Wyngaarden, who later became director of the NIH. [4] Tom had a lot of experience in biochemistry. He had worked with Wyngaarden on some of the enzymes of purine synthesis or purine metabolism.[5] Tom had published a paper with Wyngaarden and was also an M.D. [William] Bill Jones, a wonderful investigator, came to my lab as a Research Associate

even though he didn't know much about the field I was working in. I could teach these Associates the work very rapidly. They were smart. They learned very quickly on their own.

When postdoctoral fellows came to the lab, they didn't know anything about nucleic acids, or about this work; so obviously I proposed all the problems that they would work on, and I helped them with the work. After a while when you build up to a critical mass, the interaction between the postdoctoral fellows is terrific, and they help one another. The biggest problem then was to decide what to do first because there were so many things that could be done. You wanted to do only the most important things, and so that was really the initial problem. The things that had to be done were by and large obvious; and after thinking about it a long time, you see every aspect of the problem. Although these people who came to the lab had no experience in the field that we were working in, everybody understood the importance of the deciphering the code. Everybody was carried away by the problem.

From Deciphering to Determining the Universality of the Code

MN: Toward the end of the deciphering period, we asked the question, "Is the code a universal code?" and we compared the codons that we had determined in *E. coli* with an amphibian, with the *Xenopus nuriella* embryos and with a mammalian liver. We found that it was really the same code, that the code was largely universal. There were some differences that we observed in the amount of tRNA corresponding to a particular codon

in different species, but it was in the same species. Since then people have observed some "dialects" in some forms of some organisms. In mitochondria, for example, there is a dialect of the code. Almost all forms of life on this planet, however, use a very similar code. Tom Caskey was one of the major individuals who proved this.[6]

This had a big philosophic impact on me. I thought this was extraordinary, that I would look out the window and I would see a tree and maybe a squirrel sitting in the tree, and I would think that the instructions in the plant and the squirrel are really the same. It is going to be the same. I thought that was just beautiful. I knew all about Darwin and evolution. This was such a striking confirmation that similar mechanisms were operative in different forms of life that it had a big emotional impact on me. I talked it over with Tom, and he had just the opposite view. He was terribly disappointed that the code was the same in different organisms. If it had come out the way he had wanted, there would have been differences in the code in different forms of life. But we found universality in the code.

RH: Was anyone else working on determining the universality of the code?

MN: The universality was initially ours alone. I think that later on people did a lot of additional work on universality and have done in the subsequent years since then. Since I got out of the coding field, I was amazed at that.

There has been a lot of work on universality since the code was deciphered. It is a totally different picture now because in just a few hours you can synthesize oligonucleotides automatically with a machine. It is an incredible advance, I think, compared to the way of synthesizing oligonucleotides back then.

It's interesting now. I almost feel like Rip Van Winkle because we have an instrument that synthesizes oligonucleotides. You simply program the sequences that you want synthesized and you can synthesize four oligonucleotides in just a few hours. It does it all automatically. When I think about it-- that instrument accomplishes more in several hours than three or four people could accomplish in a year back in the '60s. Now they're synthesized by a completely different route, and we synthesize oligos all the time to use for many different purposes. But then you had to make all the intermediates; you had to make all of the compounds, the starting materials. You couldn't buy them.

A few years ago somebody induced me to give a talk here at the NIH in a Grand Rounds on the work that was done on the code after the code was deciphered, that is, in the 25 or more intervening years. I thought I knew all the developments because I followed them in the literature when they appeared, but I wanted to at least re-read the papers before giving this talk. So I ran a computer check of the literature, and I was amazed to see the number of papers that had been published since the code was deciphered. I was amazed at the number of changes, of new things, that had been done with the code.

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

[1]

[2] Give stetten's position at that time just to be consistent – in the link

[3] Interviews with Sidney Udenfriend, 26 October 1995 and Perola Nirenberg, 15 December 1995, by Ruth Harris, interview notes at the Office of NIH History, NIH.

[4] James Barnes Wyngaarden (1924-) received an M.D. at the University of Michigan Medical School in 1948 and served as the director of the NIH from 1981 to 1989.

[5] C. Thomas Caskey received an M.D. in 1963 from Duke University Medical School. From 1965 to 1967 he served as a research associate for Marshall Nirenberg at the National Heart, Lung and Blood Institute and continued in that Institute where he became chief of the Section of Medical Genetics from 1970 to 1971. In 1971 he started a long association with the Baylor College of Medicine where he served as chair of the Department of Molecular and Human Genetics from 1994 to 1995. From 1994 to 2000 he was senior vice president for research at Merck Research Laboratories in West Point, Pennsylvania.

[6] Caskey was co-author of several publications on termination, including "Release Factors Differing in Specificity for Terminator Codons," *Proceedings of the National Academy of Sciences* (USA), 61: 768-774 (1968) with E. Scolnick, R. Tompkins, and Nirenberg.