Transition to Neurobiology, 1965-1969

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/neuroblastoma.html

As the race to decipher the genetic code came to a close in 1965, Nirenberg sought out new scientific puzzles. Many minds were still trying to unravel the mysteries of protein synthesis, but Nirenberg's mind was on another mystery--that of the mind itself. From 1965 to 1969 Nirenberg turned his attention and his laboratory over to the field of neurobiology.

Neurobiology is the study of the brain and the nervous system. Neurobiologists seek to understand the many facets of this system by considering its development, physiology, activities, and malfunctions. Neurons, the cells of the nervous system, interact with each other at molecular, chemical, cellular, and electrical levels to form an intricate and complicated web called the "neural network". In 1913, physiologist Edgar Douglas Adrian studied the neural network and discovered that neurons transfer information throughout the nervous system by sending electrical impulses in discrete units, creating "spikes" of electrical discharge. Modern neurobiologists still study the neural network by investigating how the nervous system processes and transfers the large amount of information that is stored within the complex web of neurons.

Nirenberg's move to neurobiology may at first seem to be a peculiar shift from molecular biology, but it makes sense when information processing is considered. There are only two biological systems that process information by receiving it, storing it, and then relaying it: the DNA- RNA-protein system, which processes heritable, genetic information; and the brain, which processes sensory and mental information. The analogy between the genetic code and the brain attracted many molecular biologists who had studied genetics. For example, Seymour Benzer studied the genetics of viruses, a subject for molecular biology, in the 1940s, and later investigated the relationship between genes and behavior in the fruit fly *Drosophila melanogaster* in the 1960s and 1970s. Similarly, Sydney Brenner helped pioneer the field of molecular biology in the 1940s and 1950s but then studied Caenorhabditis elegans, a small roundworm, to examine its genetics and behavior in the 1960s and 1970s. Julius Adler also, , worked extensively in molecular biology and then moved on in the 1960s to investigate the chemical basis of motion in bacteria, a topic in neurobiology. Other molecular biologists, such as Gunther Stent, Max Delbruck, Francis Crick, and Cyrus Levinthal, joined these scientists in the molecular migration to neurobiology in the late 1960s and early 1970s.

Neurobiology offered Nirenberg the freedom to investigate a whole new field of science. He has called this period of his scientific career one of the happiest times of his life. But the transition to neurobiology and the newfound freedom it offered was not entirely free of problems. Nirenberg had made a similar transition in the late 1950s when he left the comfort and familiarity of biochemistry to enter the unknown world of molecular biology. A decade later, he was making yet another transition from the familiar to the unknown. Nirenberg also, , faced other dilemmas. Neurobiology was still evolving and experienced growing pains of its own in the 1960s; as Nirenberg perceived, entering neurobiology was "like walking up to your neck in a swamp."

One of the "swampy" areas of neurobiology in 1967 revolved around the nature of the neural code. In the 1950s and 1960s, neurobiologists thought that Adrian's spikes might carry and process the neural information in the form of a neural code. Nirenberg studied the neural code by employing the conceptual and experimental approaches to science that proved So, successful for him in his work on the genetic code. He looked for the general rules of the code: he sought to identify the basic units of information used by the system; he hunted for the underlying logic of the system; and he immediately began considering what biological systems he could utilize to study the neural network and its code.

Nirenberg considered the various facets of the neural code for more than a year, but these reflections never made it into a published form. Nirenberg did draw the analogy between the genetic code and the neural code in a draft of his 1968 Nobel acceptance speech, but he edited this material from the final version. Nirenberg also, , presented his ideas at Howard University in a 1969 lecture titled "Genetic Versus Neural Information Processing Systems", but he was only willing to endorse the thoughts as "speculations". While Nirenberg's interest in the similarities between the genetic and the neural code never evolved past the conceptual stage, these ideas helped to spark his curiosity in how the brain and the nervous system develop. This curiosity would drive much of his research in neurobiology for the next thirty years.

The Harris Interviews – 1995 – 1996

Ruth Harris (RH): You switched from the field of the genetic code to the complex field of neuroscience.
If you had to do it all over again, would you still change fields?

Marshall Nirenberg (MN): Absolutely. I would still do it. No question. After you have been in a field for about ten years, you have thought of most of the questions and many of the answers as well, and you have perspective on the entire field. The unknown beckons. What is over the hill?

I felt that at the time that I switched to neurobiology I could continue to work in the field of cell-free protein synthesis and gene regulation, which was going superbly.

There were a tremendous number of beautiful projects to work on once you had all of the methods worked out and going on in the laboratory and you had collected all the intermediates and everything else.

Even though the work was beautiful, it wasn't a challenge. Once you get things going in the field, it becomes easy because you have done all the preliminary work. I felt I could do it with one hand tied behind my back. It was easy to do, too easy. So, it was much easier for me to continue to work in molecular biology, in protein synthesis, in regulation of gene expression, than it was to go into the nervous system, about which I knew zero.

The problem with being very productive in a field like the genetic code is that you have a lot of responsibilities. You have to write papers. For scientific papers, you have to write them as fast as you can. But I like to get it right, So, I tend to write and rewrite too much, and it takes too much time. Once I start, I like to write. I do not mind it at all. But getting started—there are So, many other things I would rather do than write. So, that is a defect, I think, from the coding days when I had strict time limitations for writing and had to get papers out. I developed a kind of antipathy towards writing that is not good at all. So, I forced myself to publish and to be productive because it was necessary. But you do that at a price.

You have to understand: what do you mean by success? What do you mean by productivity? To be honest—you don't get a chance to be highly productive that often in a field. When you do get a chance like that, I suppose you should milk it for everything that it is worth because the work that you are doing is beautiful.

Getting back to the question of what is success for a scientist, I think that a person goes into science because he wants to discover things, and the search is basically the important aspect. It is the journey, not the end result So, much, which is the important thing. I think there is no doubt in my mind whatsoever that if I had stayed in the old field, I would have been more productive. There would have been more papers and there would have been maybe even better work. Although when I look back on it, I think that I achieved many of my initial objectives. I worked like hell.

You are working with other people and you have to take care of their needs, and you have to decide what to do with your time. You keep taking out without putting things back into yourself, without continuing to learn, and that is not a good situation. Always the thing that came first and foremost was to get the work out, to publish the work, and to finish the work that we were doing, So, sometimes there wasn't enough time to also, read and think.

I had been doing that for ten years by then. I had been grinding it out and had been extraordinarily productive and at some cost to myself. By “cost,” I mean scientific cost. Instead of reading material in a journal that just came in, I would be writing a paper that had to get written by a certain date. The thing that is the most fun of all is to read and to think: to read what other people have been doing and to think about these papers and about the questions that one can work on, possible questions and possible experiments.

Switching to neurobiology meant total freedom, freedom to do what I wanted to do for the first time in many years instead of just meeting my responsibilities.

RH: Which areas of neuroscience attracted you?

MN: Before I went into protein synthesis, nucleic acid metabolism or synthesis, I thought about the areas that I was really interested in and would like to work on and it came down to regulation of gene expression versus the brain. There are only two major systems in biology that process information: the nervous system and storing and retrieving memories and the genes storing and retrieving genetic information. There is a real similarity between the two of them. I didn't really know anything about the nervous system, but I was eager to learn and eager to try to understand it. It was a marvelous puzzle, and I wanted to jump into it and to see if I could do something with this problem.

I didn't know anything about neurobiology, but I was always interested in neurobiology, and So, the possibility of discovering something in an unknown area was a great stimulus. I wanted challenge, So, that is why I went into neurobiology—because the challenge was there.

When I finally decided to switch to neurobiology, it was an absolute joy. I spent a summer attending a course that was run by the Massachusetts Institute of Technology (MIT) Neurosciences Program in Boulder, Colorado. Perola and I went out there for the summer. The Rockies are just magnificent, and Boulder is just next to the Rockies, So, on weekends we could go up in the mountains. It was just absolutely glorious. It was such fun to start thinking about the totally new field of neurobiology. Every day leading people in the field would give talks in this course. Many of the leading people in the field were there. I met and listened to them.

I gave a talk on my work, they gave talks on their work. It was a wonderful experience. I felt rejuvenated by jumping head-first into neurobiology. It was as glorious a summer as I have ever had in my life, and I loved it.

Here I was going into a field that I had never thought about and didn't know much about. So, I was on a rapid, steep learning curve. Virtually everything that was said was new to me. I learned fast. I met a lot of new people. It was total immersion in neurobiology. I had an enormous number of ideas about neurobiology also, and since it was So, new, it was stimulating to me. It was a wonderful entrée into the field.

Switching fields like this is not a simple thing. There are many conflicting responses enmeshed in the decision. I certainly didn't want to fail. I wanted to succeed. When you are switching fields like that, you start from the absolute beginning. Everything that you have done, you have basically turned your back on and given it away. It takes a tremendous amount of work and effort to get set up to work in a field.

You need to work out the assays, to become familiar with the literature, to think enough about the field to have some type of orientation and understanding of the relative importance of problems and to determine which problems are doable and not doable.

RH: Did some of the people in your lab, such as the postdocs and the research biochemists, continue to work on the genetic code work while you moved on?

MN: For the first two years I tried to do both neurobiology and a continuation of the other. Basically, I gave the postdoctoral fellows with whom I was working to Tom Caskey, who was superbly qualified to lead the group. He was working on mechanisms of termination of protein synthesis.

I tried to do both things, but it rapidly became obvious to me that I couldn't do both things simultaneously. I could do one or the other. It was hard enough to do either one of them. So,

after some time I completely switched. One of the postdocs, Bruce Schrier, who had come to me to work on protein synthesis, actually switched to neurobiology with me. But all the others stayed with Tom, and they did beautiful work with Tom because everything was going well. Tom did a wonderful job, particularly with termination, and published a set of papers on termination factors. Joe Goldstein came to work with me, but I turned him over to Tom at the time because I was starting to be interested in neurobiology.

After I switched, I could devote 100 percent of my time to neurobiology. I just plunged in head-first and immersed myself morning, noon, and night in neurobiology. Initially, the lab was like a zoo. I collected all kinds of invertebrates. I was looking for model systems to use, simple nervous systems to explore.

Eric Kandel once described the work that I and a few other people who went into neurobiology did as: "The early barbarians that stormed the shores of electrophysiology and were never heard from again."

I started as a biologist. I am interested in simple organisms. I had Rhodoferax in the lab and Daphnia, little brine shrimp that you can buy as tropical fish food, and all kinds of things. It soon became obvious that one of the nematodes could be or was a very important system because it was the only system with a defined diet that had been found. You could grow them on a relatively defined diet, an axenic diet, and there were no other invertebrates with which you could do this. I spent a few years with the nematodes, getting mutants, becoming familiar with them. At about the same time Sydney Brenner started studying the nematodes, and he did it for the same reason that I did it. [1] This was before Brenner had published anything on nematodes. From a personal point of view, it was very successful, even though it was not as productive scientifically as it might have been. Although "productive" is a funny term.

Ruth Purtell from Berkeley, who had worked on nematodes for her Ph.D. thesis, came to the lab as a postdoctoral fellow and was a considerable help there. Anyway, I thought it was a glorious experience.

RH: When you started your work and you got a little grasp of the neurobiology, what was the single most important question that you wanted to answer?

MN: There were a tremendous number of questions actually. My objective was to devise simple cell systems, clonal cell systems that were useful for biochemical and electrophysiologic studies of neurons and that were able to form synapses. We got clonal cell lines that would form synapses. We studied them, we characterized them, and we found that they were regulated. We accomplished that objective ultimately.

Of course, when one thinks of the nervous system, one asks the question: what is memory? How do you store memory? How is memory retrieved? These questions are still not answered. They are still some of the major questions of all. How in the world do you construct a nervous system?

You need genes. Genes have to provide the information for constructing a nervous system. How do you specify the billions and billions of neurons and even more billions of connections between neurons? How do you specify synaptic circuits? How do you determine appropriate from inappropriate synaptic partners to make synaptic circuits? How do you build a nervous system? Those are the major questions, as far as I can see, and these questions have not been answered.

There is much information that suggests that selective adhesion is important. Studies indicate that a class of enzymes known to be involved in transducing signals from outside the cell to inside the cell involving protein kinases—enzymes that catalyze phosphorylation of certain proteins—may be involved in this process. But nobody has pinned that down exactly.

RH: Can you name some of your outstanding neuroscience postdocs and tell what happened to them after they left your lab?

MN: Sure. I have been blessed with working with some wonderful postdoctoral fellows who were wonderful before they came to my lab and they have done extremely well after they left my lab.

Al Gilman

MN: In neurobiology, there is Al Gilman, who came to my lab in the early 1970s. Al Gilman was highly unusual, because he knew what he wanted to do in the long haul before he came to the lab. He wanted to continue the work that he had been doing as a pre-doctoral fellow. He had worked on cyclic-AMP for his predoctoral research and knew exactly what he wanted to do when he came in the lab.[2] When he came for a postdoctoral fellowship in my lab, he wanted to work on adenylate cyclase or cyclic-AMP also. And that is exactly what he did.

He assayed cyclic-AMP levels of cells from different clones and characterized some of the receptors that were coupled to adenylate cyclase receptors, for example, in these clones. He continued to work with clonal cell lines and, in fact, used a defective cell line from a lymphoma.

Later on after he left the lab, he used a cell line that had a defect. It lacked one of the proteins that are required to couple the receptors to adenylate cyclase. This allowed him to devise a supplementation assay, a complementation assay that could be done in vitro to identify, to assay, the coupling factors. This cracked open the entire field of the mechanism of transduction of hormonal and transmitter-mediated activation or inhibition of adenylate cyclase. In 1994 he won the Nobel Prize in Physiology or Medicine for the work that he and his colleagues have done in this area.

When Gilman came to the lab in the 1970s, he knew more about cyclic-AMP than I did, and so, he was quite independent. We conferred a whole lot. He didn't know anything about tissue culture, or somatic hybrid cells, or mutant cell lines, various mutants, and things of this sort, which I was able to show him.

As a matter of fact, while he was a postdoctoral fellow, he hit on a way of assaying cyclic-AMP that was much easier and much faster than the only method that was available at the time.

The only method that was available at the time took about four days to do, and it required weeks of preliminary work to purify the enzymes to use in the assay because they were not commercially available. He devised a very rapid way of assaying cyclic-AMP by studying binding of cyclic-AMP to a particular protein, a binding protein. He came to me and told me about the problem, and I supported him 100 percent in doing it and gave him my technician to help him do it. He published the paper by himself

Actually I sponsored his paper in *PNAS*. It was a big help to the entire field because it was a fast, simple, rapid assay for cyclic-AMP, the first assay of this type that was available, and So, it

speeded up work in the entire field. He did all of that while he was a postdoctoral fellow, and he did that independently

At that time nobody knew why GTP was needed. There was a very important clue that indicated that there was another reaction that required GTP for coupling a receptor to adenylate cyclase that [Martin] Marty Rodbell had discovered. Rodbell shared the Nobel Prize with him and was at the NIH at the time.[3]

Al had discovered and shown that GTP is required for the coupling, but nobody understood in molecular terms why GTP was needed. This proved to be an enormous field that for 15 to 20 years now has grown to tremendous proportions and turned out to be extremely complicated. It is a baroque field with many kinds of proteins and coupling factors involved in it. It is a major area of research in biochemistry now. Al Gilman cracked it all open with this assay. It was a very important paper. It was a remarkable thing for a postdoctoral fellow to do, and he did it on his own, by himself. It was a very important contribution at the time because it simplified and speeded up the work so, much.

He was the most knowledgeable person in the lab about cyclic-AMP. After he left the lab, actually it was to continue to work on that problem, to find out how adenylate cyclase—how you have transmembrane communication between the outside of the cell to the inside of the cell mediated by receptors. While here he learned tissue culture, and he looked at the cyclic-AMP levels and responses of some of the cell lines, neuroblastoma cell lines, that we had established, to various neurotransmitters and found some that influence cyclic-AMP levels of cells. He worked with the somatic hybrid cells that we had developed in the lab. I think that proved to be very useful for his work after he left the lab because that approach and the approach of using a mutant cell line that lacks one of the factors broke wide open a whole problem.

Actually, many postdocs went on to Dallas and to Southwestern. All of these people were at NIH at the same time, and they basically brought one another to the same place. I don't know the sequence in there, but I think they were instrumental in that when opportunities came at that medical school, they alerted each other. Three or four departments are headed by people who were in my lab at the time. And they were really terrific investigators.

[11] Sydney Brenner (1927-), considered one of the founders of molecular biology, earned a master's degree in medical biology at the University of the Witwatersrand in 1947 and a PH.D from Oxford in 1954. His best known work was the discovery of messenger RNA and work with nematodes.

[2] Alfred Goodman Gilman (1941-) earned a B.A. in 1962 from Yale University and a Ph.D in pharmacology in 1969 at Case Western Reserve University. He served in the Pharmacology Research Associate Training Program at the National Institute of General Medical Sciences and in 1971 became an assistant professor of pharmacology at the University of Virginia, where he began his Nobel Prize winning research.

[3] Martin Rodbell (1925-1998) earned a B.A. at the Johns Hopkins University in 1949 and a Ph.D. in biochemistry in 1954 at the University of Washington. He conducted research at the National Heart Institute from 1956 to 1962 and taught and engaged in research at five United States and four European universities between 1954 and 1996. Rodbell served as chief of the Laboratory of Nutrition and Endocrinology of the NIAMDD from 1962 to 1985, as scientific director from 1985 to 1989, and as chief of the Section of Signal Transduction of the National Institute of Environmental Health Sciences from 1989 to 1990.