Interview with Marshall Nirenberg
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Topic: Research,Genetics,Social/Political Activism
Interview by Eric Boyle

Eric Boyle (EB): Since a lot of the Digital Manuscripts Program collection covers the latter portion of your career, I'd like to discuss briefly the general trajectory of your research from the neuroblastoma and hybrid cells and chick retina work to *Drosophila* and homeobox genes in the 1980s and beyond. Now in your interview with Jim Tabery last year you touched on your earlier work with *Drosophila* and homeobox genes. Can you discuss this transition and how or why it took place.

Marshall Nirenberg (MN): Well the first transition really took place between the time we deciphered the genetic code and the time I decided to go into neurobiology. And the logic to that was that there are two systems in biology that process information, genes and the nervous system. And I was always interested in the nervous system anyway, although I didn't know much about it. So, that was really one of the big factors that made me interested in it is that I didn't know much about it. So, that was the logic for switching from basically genes and protein synthesis in the genetic code to neurobiology. Now when you make a change like this it was a big change. It was so easy to do the work in molecular biology and protein synthesis related to messenger RNA and the code. It was like turning a crank. I thought I could do it with one hand tied behind my back. But I gave all of my postdoctoral fellows and all of the problems we had going to Tom Cassidy who was a former postdoctoral fellow. He took over all of that work so I was free then to explore neurobiology. Basically the thinking behind it is that it is really fun to explore and the only way to really discover things is to jump in and ask question and begin to explore the field. It's a risky business though. I mean you're giving up all of the things that took you a long, long time to set up—all of the procedures, the methods, an established laboratory, basically. So you do it with the knowledge that there is a lot of risk involved in doing it. Nobody wants to fail. I don't want to fail. And yet there is a real possibility of doing that. So you weigh different thoughts and ideas in doing it.

So, anyway when I decided to go into neurobiology it was a fantastic and wonderful experience because I didn't know anything. You're starting from the beginning, really from the beginning, and it was just a wonderful sense of learning, exploration, of freedom. You know I didn't have the responsibilities then because I had given away all of my responsibilities. It was a wonderful learning experience. I decided, eventually, to explore two different paths. One was neuroblastoma cells. Neurons are non-dividing cells and I thought it would be nice, it would be wonderful, to have a simple system of clonal cells lines that had the properties of neurons and which grew synapses in culture. And so I switched to, started to study, a tumor of neurons called the neuroblastoma--a mouse neuroblastoma transplantable tumor. We did a lot of work with the neuroblastoma cells. Gordon Sato, also about the same time cloned the same mouse neuroblastoma, the C-1300 that we did. And I also began to explore C. elegans, nematodes, as a simple system. It turned out that the only invertebrate that you could culture in a known

medium, a defined medium, was a nematode. There were a number of species of nematodes that had been used at the time and this was before I knew that Sydney Brenner had also picked nematodes to study. So, we worked a couple of years on nematodes making mutants and so forth. I never published anything about the nematodes and eventually I thought that both systems were very promising but Sydney Brenner was doing very beautiful work with the nematode system and I thought that I could only handle one or the other, not both simultaneously.

So I switched to neuroblastoma cells and our initial objective was to clone mouse neuroblastoma cells, characterize there neural properties, and determine whether they could form synapses. If they had sufficient neural properties, they made neural transmitters for example, to be able to form synapses and use it as a model system for studying synapse formation and information processing by neurons. We spent a lot of time doing this. It proved to be a very fruitful thing. Takehiko Amano came to the lab about that time. He was a superb tissue culturist from Japan and he was the one who basically cloned all the lines of neuroblastoma cells. And we had many, many lines of neuroblastoma cells. And then I began to set up with some postdoctoral fellows who came to the lab who wanted to work in this area, set up biochemical assays for various neural properties, like choline acetyltransferase that catalyzes the synthesis of acetylcholine, or acetylcholinesterase which catalyzes its breakdown, or a number of other assays, also some enzymes of catecholamine biosynthesis.

We also began to study the electrophysiologic properties of the cells and we soon found that with the many different clones that we had that some clones synthesized norepinephrine, whereas other clones synthesized acetylcholine. This was the first time that acetylcholine had ever been found as a neurotransmitter in neuroblastoma cells and that was exciting. Later it was found that in humans, also, some neuroblastoma cells synthesize acetylcholine. So we had both cholinergic and adernergic neuroblastoma cells and many neuroblastoma lines that would synthesize either transmitter. And we explored the properties of these cells in great detail and we found that the neuroblastoma lines that would synthesize acetylcholine could form synapses with striated muscle cells in culture. Later we found a clonal line of muscle cells that we could use with clonal neuroblastoma cells to form synapses. We could innervate every muscle cell on the plate. We found that many of the neural properties were regulated by the level of cyclic AMP in the cells. We found that if we elevated cellular cyclic AMP for a number of days, like five days, we could shift the cells from a relatively undifferentiated state to a differentiated state where they behaved like neurons. They acquired voltage sensitive ion channels, sodium channels, potassium channels, calcium channels, and this then could from action potentials. We showed that they could release transmitters and they innervated muscles cells. If you reduced the level of cyclic AMP you could reduce the effectiveness of the synapses. You could regulate the efficiency of the synapses by the level of cyclic AMP that the cells possessed. We used a number of techniques to elevate cellular cyclic AMP. Like we found that the cells had prostaglandin E-1 receptors that were linked to activation of adenylate cyclase when they were stimulated. So, we would add prostaglandin E-1 to the culture medium that they would then activate the endogenous adenylate cyclase,

which would catalyze the synthesis of cyclic AMP. You could raise cyclic AMP levels over a period of days. These effects were slowly acquired and long lived.

I've always thought we were looking at some form of memory, some type of memory, although I couldn't prove it and still can't prove it. But I think the ability to turn synapses on and turn synapses off at will and the length of time, the slow acquisition of the ion channels and the other machinery that are necessary for transmitter release and effective synapse communication, all indicated that this was a form of memory. That if you lowered the cyclic AMP levels gradually this cell became less able and finally unable to communicate synaptically. This was a very interesting type of system to work in. You could use these cells to study the biochemistry as well as the electrophysiology of the process—a good model system. And that's what my aim was, to make model systems that were useful for neurobiology.

We used another technique to rescue gene expression. Many of the cell lines were defective in synapse formation and so we fused the neuroblastoma cells to other cell types from the normal nervous system hoping to rescue gene expression for neural properties. We made somatic cell hybrids by fusing cells of different kinds and we made many kind of somatic hybrid cells that proved to have interesting properties. We found that many cell lines had specific defects in synapse formation and we characterized those defects. These are useful system, I've sent these cell lines to investigators all over the world for many years.

EB: One of the things that was most striking from looking through your papers is that you have letters from Japan and Europe, all over the world.

MN: We sent hundreds and hundreds, thousands probably, of cell lines to thousands of investigators all over the world over many, many years. They've been used in many ways so that's, I think, really been a very useful system.

One of the things we did I did with Werner Klee who was an investigator at the NIH and a superb biochemist. Werner was interested in opiates and he wondered if the neuroblastoma or the somatic hybrid cells had any opiate receptors. We screened many of the lines, actually I've got thousands of cell lines, and really it's really a cell bank. It's probably more neuroblastoma or hybrid cell lines than exist anywhere else in the world. And so we screened many of cell lines looking for opiate receptors. And lo' and behold we found one of our most well studied cell lines, the NG-10815 cells, which is a neuroblastoma-glioma hybrid cell line, was loaded with opiate receptors. Once we found that and we were able to count the density of receptors on the cells we wondered what would happen if we cultured the cells in the presence of morphine. For example, would they become addicted, would they become dependent on morphine. This led to some really interesting experiments. We found that when you culture the cells in the presence of morphine that morphine inhibits adenylate cyclase activity, so the level of cyclic AMP drops precipitously. But then gradually if you continue to incubate the cells over the course of a day the level gradually comes back to the normal level. Now, the reason that

it comes back to the normal level is because the activity of adenylate cyclase increases during this day's incubation. But then the cells are dependent upon morphine to inhibit the enzyme because if you withdraw the morphine, the level of adenylate cyclase activity shoots way up and the amount of cyclic AMP synthesized really goes way up and then only gradually falls back to the normal level. So, we proposed this dual regulation of adenylate cyclase—morphine inhibits adenylate cyclase and the cells gradually become dependent upon inhibition of adenylate cyclase and the basal level comes back to normal—and this is equivalent to withdrawal phenomenon of the opiates. Other have confirmed these findings and we also extended the findings to other neurotransmitters. We found exactly the same thing with the muscarinic acetylcholine receptors. If you treat with carbachol, it inhibits adenylate cyclase. Gradually the level of adenylate cyclase will come up to the normal level. Then the cells are dependent upon carbachol to inhibit the enzyme. The same thing with the alpha receptors. I think it is a general phenomenon. We don't truly understand everything about this phenomenon even today because we asked the question: are we synthesizing more enzyme or simply activating the enzyme? The results showed that it's mixed. Mostly you're activating the enzyme but there is some synthesis of the enzyme, increased synthesis that does occur that counts for about a quarter of the increase in adenylate cyclase activity. Three quarters of the increase is simply activation of the enzyme. I don't know how the enzyme is activated like that, that remains to be determined.

EB: That's what I was going to ask you.

MN: I don't know. And I really wondered at the time. That work really raised questions in my mind about possible treatments for opiate addiction and dependence. I never pursued the clinical aspects of the findings but I always regretted not having pursued that because I think that might have been a very fruitful pharmacologic approach to the problem of opiate addiction. That problem really interested me for a number of years.

Then I became interested in a different kind of a problem. In the sixties it was proposed by the grandfather of neurobiology, Roger Sprerry, in a theoretical article in PNAS, strictly theory, but an interesting theory, that you could label, you could give every cell in the retina an address, a molecular address with two kinds of gradients of molecules in right angles to one another. That was a very intriguing suggestion. The reason he suggested this is because it's known that in the brain there are various retinal topographic maps that topographically reproduce the position of neurons in the retina so that you can recreate a cohesive picture of the outside world. There are multiple topographic maps like this that are produced and nobody knows how they're produced, or what the molecular bases of the maps really are.

So, I thought of a way of asking: is there a molecular topographic map that exists in the retina? And to study this we used monoclonal antibodies. Monoclonal antibodies are produced with a clonal cell where each clone makes a different kind of an antibody in culture, and so we used as an antigen cells from dorsal retina, basal retina, temporal retina, or ventral retina--different topographic regions of the retina--and made monoclonal

antibodies and then made thousands of different clones, screened these clones and asked the question does this antibody recognize an antigen that is preferentially distributed in one region of the retina rather than the other retina (what Sperry had predicted, had suggested). This was a test of Sperry's prediction. And lo' and behold we found an antibody that recognized a molecule that we called the topographic that was distributed in a dorsal-ventral gradient all the way across the retina. And it was a big gradient, like a thirty-five fold gradient. We purified the antigen, the molecule that the antibody was recognizing, and found it was a small membrane protein that's present in highest concentration in the dorsal retina and lowest concentration in the ventral retina.

This was really a fascinating affair and the interesting thing to study, because the way the retina develops is concentrically, the oldest part of the retina is the center of the retina and then you have rings of neurons that are laid down, concentric rings as development proceeds. And how you form a dorsal-ventral gradient with that kind of developmental history was really, I didn't understand it, couldn't understand it at all. We also found out that the cells remember the amount of protein that they're supposed to synthesize because we could trypsanize the retina, separate the cells (when you treat them with trypsan, a protealytic enzyme that destroys the antigen) and then we would culture the cells and we would take cells from dorsal retina or ventral retina or in between and do the separating we found that they remembered how much protein to synthesize. And it didn't require cell-cell contact, it didn't require contact with cells that had less or more of the antigen. Nor was it regulated by mixing cells from ventral and dorsal retina—cells had a memory of how much protein to synthesize. That was really exciting because this was the first time a protein had ever been found that was distributed in a concentration gradient across the entire tissue. I didn't understand and still don't truly understand how a grade of synthesis or accumulation of this protein could occur, what the mechanism regulating the synthesis and or degradation of this protein was. What did I understand? What the function of the protein was. We tried to clone DNA for this protein. We purified the protein. That was quite a job, to get enough retina to purify the protein and identify the protein.

One interesting thing, during the course of this study we found a chick embryo with three eyes. Two eyes in the regular place and the third eye in the middle of the forehead and we asked whether the third eye also formed a gradient and it did. All three eyes had exactly the same gradient. But we failed to clone DNA for this protein and that was an important question because if we could have cloned it we could have identified the amino acid sequence of the protein and would have given us a tool to use, a very important tool, for further studies. For some reason we were not successful in cloning it.

At that time, soon afterward actually, there were a lot of reports of gradients of proteins that had been found in *Drosophila*. I thought that to really understand this problem you have to go to a simpler system where you have genetics that you can use. You can use genetics as a tool. *Drosophila* has been studied for a hundred years almost, ninety years, and there is a tremendous amount of genetic information that is known and wonderful genetic tools that can be used with *Drosophila*. And that's the reason I switched to

Drosophila. Plus the fact that I saw a paper by Michael Levine and his co-workers on evenscate, which is a homeobox protein that was distributed quite remarkably in some neurons in the developing embryo and not in other neurons (highly specific expression of the gene regulation of homeobox genes), genes that bind to DNA and recognize the sequence in DNA that turn genes on or off, so they're gene regulators. So this is an important class of genes and to find them quite specifically distributed in specific sets of neurons was quite a remarkable observation. At that time there were seventeen homeobox genes that were known, that had been found in *Drosophila* and it was a burgeoning field of study.

I was really intrigued by this expression of a specific gene regulator in a specific subset of neurons because we had been trying to find things like this with monoclonal antibodies as a tool and here it was in *Drosophila*. I had never worked with *Drosophila* before but when Yongsok Kim came to my lab as a postdoctoral fellow immediately after he got his Ph.D. in Korea, I suggested to him that we look for new homeobox genes in *Drosophila*. I designed a whole set of all the nucleotides that could be used as hybridization probes to screen the library for new homeobox genes. These were oligonucleotides to highly conserved regions of the homeobox. The homeobox is a hundred and eighty base pair region that encodes a conserved sequence of sixty amino acid residues that fold in a characteristic way that have a turned helix confirmation. And this is the part of protein that actually binds to DNA and recognizes the sequence of DNA. And so they are relatively constant, conserved regions within the homeobox. And so Yongsok is a superb scientist and in a very short time he had discovered four new homeobox genes in *Drosophila* which he called NK-1, 2, 3, and 4. And they all proved to be extremely interesting.

I've continued to work with NK-2 because first of all it was distributed primarily in the developing nervous system and it was expressed so early that I thought it might turn on neural development, might even be the first step, the commitment step and that later was shown to be true. It turns on neural development in the ventral part of the neuroectoderm in a stripe, an anterior-posterior stripe of cells, and it initiates neural development in this stripe. It's really interesting the way the nervous system is formed in *Drosophila*. There are four anterior-posterior stripes which are next to one another, of cells, and they all originate differently, in different mechanisms of origin. Eventually they form neurons that will form the ventral nerve cord, which is like the spinal cord in higher organisms. It's a really interesting story how they arise, how they regulate one another. NK-2 initiates neural development in the most ventral portion of the neuroectoderm. Another homeobox gene, a different one, initiates neural development in the intermediate neuroectoderm. Still a third homeobox gene, a different one, we don't know what initiates neural development in the dorsal neuroectoderm but MSH is required for specification of the identity of some neuroblasts in the dorsal neuroectoderm. So, there are four different genes or proteins, they're all gene regulatory proteins, that are required to initiate neural development to form the ventral nerve cord. In higher organisms, in the early neural development, the homologs of these genes are found in the same relative position during early development in what gives rise to the spinal cord. I think the basic

mechanism has been conserved in evolution. So, anyway, that's how I got into *Drosophila*.

EB: It seems like *Drosophila*, again, is giving you that model system that you were talking about earlier.

MN: That's right, that's exactly right.

EB: So, you mentioned sequencing a couple of times with the oligonucleotides and again some sequencing was involved with the homeobox genes themselves that were discovered in *Drosophila*.

MN: Yes, right.

EB: So, I'm wondering if there is a relationship between this sequencing work and what we've been hearing so much about in the genome project and their sequencing in the 1990s.

MN: Well, I think the genome project and the project on other organisms are extremely important. My goodness, the only instructions for how you build an organism are really present in the genome. The *Drosophila* genome has been sequenced also. This information is stimulating research in all directions, all fields, so it's really an amazing time right now where there are thousands and thousands of genes that have been discovered and in many cases nobody knows what the function of these genes are. So, the functional genomics is really important now. Currently we're screening the *Drosophila* genome using RNA interferons which is a new method which will give you the equivalent of oligonucleotides by injecting double stranded RNA corresponding to a specific messenger RNA. This results in the destruction of that messenger RNA and it looks like a mutant phenotype then. We're looking for genes that are affecting the development of the nervous system and we've found quite a few genes thus far.

EB: How do you feel about some of the forecasts that have been made for biomedical applications in genomic research? Do you think the distance between the research that is taking place now and those biomedical applications to come is perhaps greater than some people promise?

MN: Well, when you're doing basic research, to translate that into useful products that have therapeutic usefulness takes a lot of work and a lot of effort. There's a big gap between the two. But I think, as I said, with the sequencing of the genome that information is available now is stimulating work all across the board. Virtually everybody's work is stimulated by it, by the availability of all this information on the genome, and so I think that this is having a very large effect on research that is being done currently throughout the world. And it will continue to do so.

EB: Another interesting parallel that I think there is between your own work and some of the discussion related to the genome project is these concerns about the direction of research and potential ethical and moral implications, reminiscent of your editorial from *Science* in 1967. One of the striking things about recent genetic research seems to be, again, these ethical questions. From what I've seen, you've taken a firm stand on some of these key debates in recent years. In 1988, you signed a letter against human cloning from the American Society for Cell Biology, to the President and Members of Congress. In 2001, you also supported a statement in favor of stem cell research to George W. Bush.

MN: Absolutely. I think that stem cell research has tremendous possibilities for future research. It's not quick. Progress won't be tremendously fast, but in the long run I think that it holds enormous promise for future research. And I think that Bush compromised on this by allowing research to be conducted with federal funds on the cell lines that were already established at that time. That's not enough by any means. I think that that kind of restriction ties the work down tremendously. We need many, many more cell lines. First of all, there aren't sixty cell lines really available and useful, which he thought that there was at the time. And you need many more cell lines. I think that's an artificial restriction that should be done away with. And I think that this approach, using stem cells, offers a lot of hope as potential therapies for various kinds of diseases—Parkinson's disease, for example, Alzheimer's disease, even repair of heart attacks, infarcts, that kill cardiac muscles cells, and other things as well. Ron McKay here at the NIH has shown that he can culture pancreatic cells that will form islets and will release insulin on demand, although at a much lower rate than is found in mice. But it works. This has to be improved, the efficiency has to be improved, even for diabetes. For repair of all kinds of cells, potentially, they could be done by this stem cell research. But the therapies will be a long time coming, it's not going to be a rapid crack or something, in fact I think it is going to be a slow, gradual, incremental thing. But I do think it has tremendous promise. And I do think that research should be pushed and that the government should support it, without the restrictions that are currently on that. In terms of human cloning, nobody I know is in favor of human cloning. I think that's a very bad approach on the instinct and I don't know anybody who is in favor of human cloning.

EB: Could you talk a little about the difference between human cloning versus stem cell research.

MN: Sure, I mean to get stem cells you take very early embryos that are only a few cells, very small, small cells, very early, early embryos and these are usually obtained as a byproduct of *in vitro* fertilization therapies typically. And most of them are ordinarily destroyed. They are routinely produced and most of them, the ones that aren't used for *in vitro* fertilization to help parents that can't have children, are destroyed. Normally. And so to take some of these tiny embryos consisting only of less than a hundred cells, fifty cells or something like that, that would ordinarily be destroyed and could be used for research purposes, stem cell research. Stem cell research is trying to use cultured cells and allow them to differentiate in different ways that they wouldn't normally differentiate, to make muscle, or nerves, or pancreatic cells, or what have you. To find

out all the steps that are needed for normal differentiation of the cells you need local hormones, stimuli of various kinds, and many other reactions that we know very little about right now. Basically, you're asking how do you form an embryo. What are the molecular reactions that occur between the first cell and the final baby that is forming, the entire organism. There is a tremendous amount of unknown information in there that really has to be obtained to have effective stem cells which could ultimately be used for replacement tissues. You know, I think that whether the work proceeds in this country or elsewhere in the world it will proceed. It's going on in other countries right now that don't have the restrictions that Bush has placed on stem cell research in this country. I think the United States should be the leader in this work instead of being unduly restricted.

EB: Another interesting thing that I think pops up that is related to a lot of these ethical or moral questions related to science specifically, but maybe exist on a broader level in some of the correspondence in the collection at DMP, is that you seem to receive a lot of letters from a lot of different organizations supporting a lot of different causes—humanitarian organizations, animal rights organizations, human rights coalitions, things like this—requesting your support. I was wondering if you could talk a little bit about your role, or participation, or support of some of those groups and what some of your ideas are about that relationship between scientists and social or political activism.

MN: Well, I think I've always tried to support causes that I believe in, that I think are good causes. So, when I receive requests of this kind, or to join with others in favor or opposed to specific ideas or things, I always try to participate. And I think it's important to do so. I normally receive lots of letters from various organizations to support different things. Those that I believe in I always support, I always try to lend my support. I think that's important to do that. Some people I think, like Linus Pauling, for example, who was very outspoken in trying to put the ideas that he believed in forth to the public, and I think that's really important to do this. At the time that he was doing it, for example, concerning the radioactivity and the bomb, things of this sort, he took at that time what was really an unpopular view and publicized the reasons why he believed in what he was saying. And I think he did a tremendous service to the country and to the world actually by doing this. I think it's very important to do it.

EB: Do you think, possibly at times there have been expectations that might have been put on you as a result of your being a Nobel laureate as far as your involvement in these types of things.

MN: Well, sometimes. I mean sometimes I get requests that are completely out of my expertise and if I really don't understand the topic then I won't either join it or oppose it because it's really truly out of my area of expertise. But some of the questions really are social questions and I don't think anybody can say that they're right all of the time. I mean I know that I've been wrong some of the time but you do the best you can.

EB: Do you think this role of being the expert or having an expertise is the only reason why people sometimes look for the support of scientists in particular, or is there a greater sense of responsibility that you feel a scientist has outside of just expertise.

MN: Well, I think that if you're knowledgeable about a particular area, more knowledgeable than most people about this area, then you should use your knowledge to impart that knowledge to the public and also it may be necessary for politicians to understand your thoughts and the reasons for your thoughts, for your particular stand on things because I think that studying science you have special knowledge that maybe the politicians don't have that would be useful to them. So, that's the reason to support or oppose particular projects.

EB: One of the things that is impressive about the body of work that is represented in the collections is how you are able to balance your responsibilities as a lab chief, as a practicing scientist, as a figure who is involved with work within the community, and also different humanitarian organizations.

MN: Well, there is never enough time to do everything I think you want to do and you have to make priorities, you have to set priorities. But within the constraints of time, certainly the bulk of my time is spent in trying to do research in the lab and in working within the lab but these other things sometimes don't take much time and if they're useful we try to be useful and we try to be helpful wherever we can.

EB: Great, thank you. It's been very helpful. I really appreciate you taking the time to meet with me today.

MN: Thank you.