Interview with Phillip G. Nelson
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Topic: Collaboration with Marshall Nirenberg
Interview by Jim Tabery

Jim Tabery (JT): What did you think of Dr. Nirenberg's work on the genetic code?

Phillip Nelson (PN): His findings were of a somewhat peculiar sort in providing a lightning flash of insight and an instantaneous solution to a profound problem. There have been many big discoveries, but it seemed that his was the most comprehensive and clear-cut solution to the most fundamental problem of 20th and now 21st century science. That's my reading of it. I'm not a molecular biologist, but it has that for me: there had been an enormous gathering of evidence but with just this one set of experiments he showed clearly and decisively the nature of the code.

JT: When I look over the work that Dr. Nirenberg has done both on the genetic code and on neuroblastoma with you, it seems that he was very interested in finding simple model systems that could get those decisive answers.

PN: Right. He liked things that didn't require statistical analysis. I'm sure you've heard this from other people as well. It's not that he didn't use statistics (standard deviations and so forth), but the phenomena he liked to deal with were either on or off, black or white, qualitative things. This was to ensure that you weren't dealing with contributory, minor modifiers but that you were really as close as possible to identifying causative phenomena.

I think that was his motivation in going to the neuroblastoma. One of his students, Nick Seeds, wrote a paper considering whether or not the neuroblastoma system was the E.coli for genetic neurobiology. The idea was that you could dissect in genetic terms the processes that were going on with neuron generation. That didn't turn out to be the case. The neuroblastoma system was a complex one that was very polyploidal. It just wasn't a clean, all-or-nothing kind of system because there was heterogeneity among the cells. Subsequently, the genetic approach has proven to be indeed the key to a lot of progress in neuroscience, but it's been more a matter of producing mutants in drosophila or mouse or other organisms. So, somewhat paradoxically, the whole organism has turned out to be a cleaner genetic system, with the effort there going to finding simple and single dimensional aspects of the phenotype which are produced by a very clear, clean, single gene knockout or alteration of gene expression. The trick has been to get clear-cut correlates of what it is that that one gene is doing, and that's been very successful. As the general neurosciences have progressed we have a number of nice systems: factors for growth, mechanics of axonal elongation, markers for various kinds of cell death, and markers for neurodifferentiation. Analysis of these sorts of phenomena has generally progressed very well, and this information has been used by the genetic manipulators of the organism to produce a genetically based developmental neurobiology. And Marshall has been very active in that area as well.

The neuroblastoma system was one of the first he studied, but he also had roundworms in the lab at the same time that he was setting up the neuroblastoma.

JT: He said that he started with nematodes and neuroblastoma, but it was eventually too much to do both, so he had to pick one. Brenner was working with the nematodes, so he went with neuroblastoma. When did you first meet each other?

PN: I was thinking about that after you called. It was 33 years ago. We were out at a summer symposium put on by Frank Schmitt in Boulder, CO (NRP). At the time, our son was just twenty months old, and he's now 34, so it must have been 33 years ago. I remember quite well that we were standing around after one of the talks, and Marshall and Eric Kandel were there in a little group, and Marshall said, "I'm really getting interested in some electrophysiology. I want to get into neurobiology." And he asked

Eric if he knew any electrophysiologists, and Eric turned to me and said, "Well, Phil's just down the hall from you in building 10!" And that started the interaction. We got together when we got back to Bethesda.

JT: And then you soon moved over into another building, didn't you?

PN: We were in Building 10 together for a while, and then I went over to Building 36. Marshall subsequently came over to 36 as well. We continued that collaboration for a number of years, with a number of co-workers.

JT: What was the collaboration like? How did you work together?

PN: Marshall was doing the cloning and purification of different cell lines, and then got into production of hybrid cells with John Minna. Takahiko Amano was doing the cell cloning, so there was a variety of cell lines that were intended to be used as separate phenotypes. They wanted to be able to do a genetic analysis to determine the basis for electric excitability, cholinergic expression, etc. The hope was that they could be used as specific synapse formers. So he had this large amount of material generated by tissue culture techniques, and was doing a number of neurochemical studies on them. I then started working with electrophysiology with the major goal being to get synaptically competent lines, so that the process of synaptogenesis could be observed and analyzed. That was really the subject of our interaction. Some of the people in his lab worked with me, and some of the people in my group worked on the electrophysiology (John Peacock, and a number of others from my lab worked on those experiments).

An interesting sidelight for me arose when we were trying to produce the genetic diversity with various hybrid cell lines which included some fibroblastic cell lines also. As part of the study of those lines, we found one very characteristic response in the non-neuronal cell lines, which then segregated and was seen partly in the hybrids and partly not in the hybrids. That research has led to investigation of the non-neuronal phenotype, which turned out to be quite interesting.

JT: Did you pursue this in your research?

PN: A ways, and then it was picked up by some others. I think the phenomenon is clearly seen in the oscillatory calcium transients in a variety of cells, but we didn't push that direction of the work to its full biological capability.

We jumped the track then to primary cell lines when Gerald Fischbach was in the lab, and he and I started working on primary muscle. We would make a culture of muscle cells from fetal chicks or mice, and then he started working on the spinal cord to go with the muscle cells to provide a synaptic system, and that worked. And then I went ahead with the neuroblastoma and muscle and was able to produce some synapses there. A lot has been done with that preparation of a neuroblastoma cell line in conjunction with either another neuronal cell line or muscle cells which can form cholinergic or other kinds of connections. But then I started to concentrate on the primary neuronal material, and that was where Marshall and I separated because I started to work on that more exclusively, and he continued with the cell lines and other preparations.

JT: So what were the years of the collaboration?

PN:. Roughly, the late 1960's (1967/1968) up to the late 1970's.

JT: What was neurobiology like in the 1960's?

PN: Well, there hadn't been nearly as much genetic dissection of the nervous system; we were still studying various aspects of plasticity. Long term potentiation (LTP) came in during that period. Also, a

lot of work in my area of neuroscience was occurring on modeling individual neurons to get their electrical characteristics, network analysis was coming in too, and there was a lot of developmental work. Richard Sidman and other people were starting that work by cataloguing genetic variants of mouse lines. Kandel was studying various forms of plasticity in the mollusks. Ron McKay, now here in the Neurology Institute, and others were starting to use neuronal material to produce monoclonal antibodies. A lot of that work was done in Marshall's lab as well. The idea was that these antibody systems would prove to be powerful probes for specific molecules, and they would permit characterization of neurons, and would also potentially be used as probes for disrupting function: if you block a function with a given antibody, then that would be a clue to what the molecular basis for that function was. Again, that's proven to be a major plus in enabling identification of subsets of certain neurons. Thomas Jessell and many other people have used that very productively. But I think it's fair to say that it has not proven to be as powerful a probe for neurological function as has the genetic dissection methodology.

JT: Dr. Nirenberg was one of several from molecular biology who made the migration to neurobiology. Do you think that they were at all influential in taking neurobiology to the genetic dissection phase?

PN: Oh yes. That's absolutely true. Benzer and his drosophila work have obviously been hugely influential. Maybe the even more important thing was that fundamental approach—the view that you can analyze things, and the notion that there is going to be some discreteness. I think many of us more traditional neuroscientists, had a feeling of the complexity and the difficulty of analyzing neural function, and that's sort of coming back to some extent. But perhaps we didn't appreciate the power of these tools and just how far you could get with them. Now, whether they're going to be enough is another question. I think that they probably aren't. So there will be some additional kind of research that is more integrative and allows for the ability to accept small, incremental changes as coming together to produce the total phenomena. But there is no doubt that these discrete techniques and approaches have enormous power.

JT: So the reductionism was an important step, but it can only get the science so far, and now we're coming upon a whole new set of problems?

PN: Maybe. I'm not sure that's established, but that's my bias. Now, I was receptive to reductionistic approaches. I started out studying spinal cord physiology on intact cats, and went to the tissue culture because I wanted a simple system too. I saw just how difficult it was to get at what was going on inside the intact system. With the new techniques people like Thomas Jessell and Marc Tessier-Lavigne have made tremendous progress in the intact systems as well. But at the time I switched over, the central nervous system was pretty opaque and didn't allow really mechanistic analysis, so the notion of tissue culture was enormously appealing to me because you could get at and see the preparations manipulated directly.

JT: Were tissue cultures being done in the late 1960's?

PN: Some beautiful work was being done by Margaret Murray and Stanley Crain and colleagues, for instance. I think my lab, with Gerald Fischbach, and Marshall's lab were instrumental in establishing that these systems could show a wide and interesting range of behavior, including synaptogenesis. They could show the full physiology and morphology of the neural tissue. So that was part of the excitement of seeing how far these could go. Gerald Fischbach and I had done some developmental studies on the muscle, and Gerry started using some preparations that had been developed by Moscona and used in morphological studies. He didn't start from scratch, but not much had been done, and there had been nothing physiological involving synapses. Gerry was interested in whether these preparations things could make synapses or not. He took the muscle and dissociated spinal cord, and he would grind up the spinal cord, strain it through lens tissue, and wound up with just a bunch of single cells, Then he'd put

the cells in a culture dish. One night he came running out of this little dark room where he did his physiology yelling, "They're there!" He'd seen these synaptic events. There was a lot of significance implicit in this because it showed that this drastically reduced system could exhibit what anyone would say was an interesting and important phenomenon, namely synaptogenesis.

I had the same reaction with the continuous cell lines. Here was a tumor that had been growing and being transplanted in various mice for 30 years. It had been discovered in the 1940's. So you get these cells out and put them down in culture and then treat them with cAMP or other differentiating agent, and you're looking for synapses. We'd been looking very hard unsuccessfully for a long time, but when we put the cholinergic line together with some muscle we found for the first time physiologically functional synapses, and that was very exciting. Marshall was very excited by the initial findings that these cells were electrically excitable. At that rather primitive stage, the notion was that the neuronal signature was an action potential, so it had been far from obvious that these cell lines that had been growing and multiplying could differentiate this way. I think he saw the possibility of dissecting these processes.

JT: Is tissue culture work fairly common now in neurobiology?

PN: Yes, it is widely, widely used. Tissue culture doesn't exactly have a controversial aspect, but it does have its opponents. It's now settled down to a pragmatic discussion about the degree to which the *in vitro* system will capture the phenomena you want to look at. I've been studying tissue cultures for 30 years now, but it is true that some processes don't get expressed as much in culture as they do *in vivo*, so you have to realize the limitations. It's an indispensable tool, but it has to be used with some degree of restraint and modesty. Getting hubristic about it isn't a good idea because you can be lead seriously astray by it. It's not a shortcoming; it's the fact that the full expression of a phenomenon probably relies on many clues in many situations. The molecular biologist may say that there is one causal factor that can be sorted out, which may or may not be true. But, probably for many things, many causes produce the same effect or participate in producing a well-balanced, stable, non-oscillatory, well-regulated effect. And some of those causes may be absent *in vitro*, and then you see some distorted form of the phenomenon, so you just have to keep that in mind.

JT: How did you get into neurobiology?

PN: I had just finished medical school at the University of Chicago, and my wife was a year behind me. I had been interested in physiology all through medical school, so I stayed during her final year and worked with Julian Tobias, who was also a molecular neurobiologist. He had wide-ranging interests in many aspects of nature and science, particularly the nervous system, and the nervous system was just one of those very interesting things. I presented my thesis work at a FASEB meeting, which was kind of the only biology meeting in those days. Then Karl Frank, who was here at NIH, offered me a job. About that time, the Vietnam War was raging. Frank's laboratory was excellent; he was doing pioneering work in intracellular recordings, so I came to his lab, and it was very rewarding.

JT: Was this in the Child Health and Human Development (NICHD)?

PN: No, that was in the Neurology Institute at the time. It was the National Institute of Neurological Diseases and Blindness. And it was in the Laboratory of Neurophysiology, which was jointly sponsored by Mental Health and Neurology. I think Seymour Kety was the overall head. Wade Marshall was the head of the Laboratory of Neurophysiology. Eric Kandel was there, Alden Spencer, Stanley Rappaport, Ichiji Tasaki, and Walter Freygang, and a lot of other very good people. So it was a very intellectually rich and personally rewarding place to work.

JT: What years were you there?

PN: I came in 1958 and switched over to Child Health in 1969. I had, meanwhile, moved into building 36. I maintained a lot of the contacts in the Neurology institute, and Marshall came over, and so we had a lot of traffic up and down. Mark Fishman came to work in his lab and then spent a year or so working with me on some of the projects that I'm still continuing.

JT: And then what brought you over here to building 49?

PN: Well, this was opening up. There had been a drive to get a building for the Child Health Institute. They were going to build it over in the north west corner of the NIH over by the firehouse, but then politics got in the way of that, as I understood it. Then the animal care facilities for primates desperately needed a new building because the facilities in building 10 were inadequate, and I think a good effect of the animal rights people came from their putting pressure toward acquiring good facilities for primates. This building was designated for that purpose and for the Child Health Institute. So there was some good space available, and with various and sundry space manipulations we got here.

JT: To wrap it up, when you look at the research collaboration between you and Dr. Nirenberg, what do you think was your most important contribution to neurobiology?

PN: Well, I think the clonal analysis approach has been very widely used. And the PC-12 cell line has been used by many neurobiologists for studies of a broad range of problems. Lloyd Greene developed that in Marshall's lab.

JT: What is the PC-12?

PN: PC-12 was a cell line isolated from a tumor. It turned out to have some very interesting properties, a primary one of which is that if you have them growing and treat them with nerve growth factor (NGF) they differentiate tremendously. They develop very long processes, and they'll make synapses and do all sorts of things. So it's been an extremely good model system for studying NGF action. It's been used by Gordon Guroff, who was here in the Child Health Institute, and many others too. I think that Marshall's insight towards this kind of approach, while it didn't lead to the total revolution that he thought it might, has been extremely useful. And that notion of genetic dissection of elementary properties is very strongly captured by the clonal analysis, and that's been part of the culture of being able to ascribe particular neurobiological functions to individual genes and their products. That's been a major thing. People in his lab were also involved in some of the positional cue molecules that specify interactions in retina. And I think it was no small part due to the association with Marshall that we got involved in the *in vitro* approach, and that primary neuronal cell cultures (Gerald Fischbach and others in my lab) have been extremely useful. So, Marshall was one of the driving forces in that whole reduced preparation and genetic analysis approach

JT: But now you are starting to see the pendulum swing back the other way towards the original approach to the whole organism?

PN: Well, I wouldn't say that it's swinging back exactly. The genetic approach is going to continue. But there are other sorts of scientists who want to look at neurobiology from the stand point not of simplicity but of how you deal with complexity. And that's not just neuroscience; it's science in general. I think that there's increasing interest in that approach as well. But I don't think it will supplant the more discrete approach.

JT: In just the last few years the NSF has put together a whole task force on biocomplexity, so there obviously is a major interest in what's going on.

PN: Well, it's not either or. In fact, one of the people in the lab here (Doug Fields) has become interested in using the gene chip approach: to look at up to several thousand genes. He's continuing with some studies along this line but using a reduced system where he can take a uniform cell type, stimulate it with varying patterns of electrical impulses, and then look for genes selected by their responses. So he's still looking at the molecular level, but he's also attracted by the notion of looking at complex responses. I don't think that's atypical. But it's not easy to do.

JT: So the question now is how do these separate approaches become integrated?

PN: Right. I think that's it: How do you use this discrete information you get from the gene manipulation approach on the problem of a more complex, balanced, multi-determinate, total organism? I wonder how Marshall would feel about it. Did you talk to him about it?

JT: I didn't bring it up. Do you still keep in touch with him?

PN: A little bit, but not much. I see him occasionally, but we're not interacting on a real scientific basis anymore.

JT: Well, thank you Dr. Nelson. This has been extremely helpful.

PN: My pleasure.