Dr. Alan Peterkofsky Oral History E

December 14, 2023

Higingbotham: Good afternoon. I am Haley Higingbotham, an Archivist at the Office of NIH History and Stetten Museum. Today is December 14, 2023. I am joined by Dr. Alan Peterkofsky. This is the fifth and final session of our oral history series on his life and career at NIH. Take it away, Dr. Peterkofsky.

Peterkofsky: Okay. Hello. I'm going to try to conclude this series by bringing you from a period of about 1975 until later on, past 2000. To give you an idea of what my status was around 1975, at that point I had been a Deputy Lab Chief in the [Marshall] Nirenberg lab for a while and was assuming considerable administrative responsibility—which took up a bit of my time and was not something that I really preferred to be doing. I had also gotten to a point where there were numerous postdocs coming to the lab. I had to spend time mentoring them, and I was spending time going to meetings and writing papers. At that point, I really did not have the time to personally get into the lab and do experiments myself, which I really missed doing. But eventually that changed. To start with, I should point out that essentially throughout my scientific career at NIH, I had been focusing on studies in bacterial systems, and there was one interlude in which I started doing experiments with animal systems.

I'll give you some background on how this happened. I had been interested in studying protein modification for a while and got into a study of this model system using this modified tripeptide—which, as it turned out, was a brain tripeptide that was composed of three amino acids. It was glutamine followed by histidine followed by proline. The interesting aspect of that tripeptide was that an amino-terminal glutamine was actually modified to make a blocked amino acid, which was called pyroglutamic acid. The third amino acid, the proline, was modified by being proline amide. We thought this was just an interesting model system to be studying. In terms of looking at that, in the extracts of the brain, it turned out that we discovered that that n-terminal pyroglutamic acid was clipped off by some kind of a peptidase, which would have left the remaining two amino acids, the histidine and proline. In the course of examining the nature of the dipeptide product, it turned out that the histidine and proline had formed a unique cyclic dipeptide of histidine and proline, which we called histidyl-proline diketopiperazine. Initially we assumed that this was a degradation product of that tripeptide, which was an active hormone, but we started to look at that and discovered, surprisingly, that that dipeptide had some activity in the brain. The first observation that we made in rats was that the peptide actually had an inhibitory effect on the effect of alcohol on rats. Essentially the alcohol tended to make them drunk. Then the dipeptide inhibited that effect of alcohol. That stimulated our thinking that this diketopiperazine was not just a degradation product of the tripeptide thyrotropin releasing hormone, but actually had some activity in its own right.

The consequence of reporting this new diketopiperazine as something that was active in the brain was that it began to attract a bunch of postdocs coming to my lab that wanted to work in that area. We continued to study that. One of the studies that we did was to see the effect of the original thyrotropin releasing hormone as well as the diketopiperazine on other kinds of activities. One of the things that we tested was the thermoregulation effect in rats. We found that this original thyrotropin releasing hormone and the diketopiperazine had opposing effects, so it seemed like there was some kind of a balanced regulation associated with this diketopiperazine compared to the original thyrotropin releasing hormone. In the course of doing these studies, when we made an antibody to the diketopiperazine and were able to measure whether there were normally occurring levels of the diketopiperazine in the brain, surprisingly, it turned out that there was something like three times higher concentration of the diketopiperazine in the brain than the tripeptide thyrotropin releasing hormone. This was

an active principle. One of the studies of another one of the numerous postdocs that came to the lab was that the diketopiperazine had an effect on cyclic GMP levels in the brain. The effect of ethanol, when you treated the brain with ethanol, attempted to decrease the levels of cyclic GMP, but the diketopiperazine blunted that effect and tended to stop that. The other thing is that, in the brain, one of the major regulatory activities that could be measured was ATPase, the degradation of ATP. It turned out that the diketopiperazine would inhibit the ATPase as well. The general conclusion was that this almost accidental discovery of diketopiperazine as an active principle was that it was playing a significant regulatory role in brain function.

Various people participated in this. The first person who had been participating in the original discovery of that was an Indian scientist named Chandan Prasad, who eventually went to St. Louis and subsequently to Texas. He spent his entire career following up on this and studying the biological activity of this histidyl-proline diketopiperazine, so it's been very, very beneficial to him.

There were a number of postdocs that came from Japan, that went back to Japan, and some of them studied diketopiperazine as well. One of the other people who participated in this study was an Israeli scientist whose name was Yitzhak Koch, who eventually went back to the Weizmann Institute in Israel and eventually became the director of neuroscience there. Altogether, it was very nice sentiment that lasted over a period of 15 years, but at the same time, we were doing other kinds of stuff.

The important thing that started a new direction in my lab was taking advantage of modern molecular biology techniques. Because we wanted to study mechanism and structure of proteins, we made a new vector virus that was able to make large amounts of protein and provide a way to do relatively simple purification of these proteins. We started this around 1990 or so. The unique feature of this vector was that we could clone genes—any gene that we were interested in—into a specific location in that vector and put that into E. coli. Then, if we grew the E. coli at 30 degrees, where the bacteria grew slower than normal, there was no expression of the protein of interest that was cloned into a vector. But when we then shifted the temperature to 37 degrees, it initiated the rapid expression of the cloned gene to give the protein in large quantities, even if the consequence of the hyperexpression of the protein created a toxic effect on the bacteria and killed them. Over a period of a couple of hours, it didn't make any difference whether this expression that created an unusual situation in the bacteria led to death. We nevertheless had a collection of bacteria that we could harvest and demonstrate that it had accumulated a very large amount—sometimes something like 40% or 50% of the total protein in the bacteria was that one protein of interest. Then we could collect those bacteria, break them, and get an extract where it was relatively easy to purify the proteins, because the protein of interest now corresponded to a very significant part of the total protein.

With that in hand, we were able, for the next 20 or so years, to take advantage of this kind of a situation where we could purify a protein of interest and study it. We used this to study proteins like the phosphotransferase system (PTS) in E. coli, as well as another kind of system that was called the alternate or nitrogen phosphotransferase system that I'll talk about in a little while. And so, with that in hand, I should point out an interesting participant in that study. During that period of time, there was a student at the local high school who came to spend a summer vacation working in the lab, and she participated in this project. Her name was Natalie Kuldell, and her father was an M.D. who had a significant position at NIH as the director of studies of orphan diseases. His interest in paying attention to his daughter was that he imagined that she would follow in his footsteps and go to medical school, get an M.D., and do something useful with that. It turned out that Natalie got so excited by doing research in the laboratory that she ignored her father's direction and pursued a path to a Ph.D. degree. She's been very successful, and she has made an organization that stimulates young scientists to get an introduction to studying bioengineering.

Higingbotham: That's amazing.

Peterkofsky: Yeah. Right now, she is the CEO of a foundation that's called BioBuilder. I've kept in touch with her over the years, and she's been very happy and very successful in that, which I assume is an offshoot of her participating in that project. That's a nice kind of story.

One of the things that is useful to point out is that the cyclic AMP and E. coli is generally used to participate in turning on the expression of various chains that require a particular factor called the inducer. There is a protein that's called the cyclic AMP receptor protein, sometimes referred to as CRP. This protein has something in the vicinity of 200 amino acids. When an inducer is present in a cell, then the inducer stimulates the binding of cyclic AMP, which then—in this complex cyclic AMP bound to the receptor protein in the presence of inducer—stimulates the expression of certain genes. One of the projects that was carried out by a postdoc in the lab at the time was to purify a mutant form of this cyclic AMP receptor protein that had the capacity to stimulate gene expression even in the absence of cyclic AMP. We interacted with a crystallographer to get a crystal structure of that mutant form of the cyclic AMP receptor protein. Her name was Irene Weber, and she was a crystallographer working for the National Cancer Institute in Frederick, Maryland. It turned out that mutant form of the protein had the position of arginine-144 substituted by another amino acid, threonine. That was a nice, tight study that was carried out in the process of my research and various things.

The other thing that I'll turn to is a number of studies that were done on the adenylate cyclase, which is the protein that is responsible for making cyclic AMP. The capability to make large amounts of adenylate cyclase with that vector that I described earlier allowed us to do a lot of different studies on that. I'll just mention that there was a postdoc in the lab who concentrated on looking at the effect of various mutants on the activity of adenylate cyclase. One of the things that she found is that this adenylate cyclase protein had a change in the capability to bind ATP if you made a mutation at the 60th position, which was occupied by glycine. Glycine 60 was necessary for ATP binding. Another one of the studies that this postdoc did was showing that the lysine residue at position 196 was absolutely essential for activity. The very important observation that was made was that adenylate cyclase activity could be stimulated in the presence of certain nucleotides. One of the studies that we did revealed that the stimulation of adenylate cyclase activity was associated with an effect by proteins on the phosphotransferase system. That shifted a lot of the interest in the laboratory away from direct studies on adenylate cyclase to studies of the transport system called the phosphotransferase system. The kinds of studies that we did involved various proteins of that system.

I should just remind you that the phosphotransferase system was connected with a bunch of different proteins in a consecutive chain. The way the system works is that there's a big protein that's called enzyme I that has a molecular weight of something like 60 kilodaltons protein of about 600 amino acids. That protein has the capacity to become phosphorylated when it's exposed to the phosphate donor called phosphoenolpyruvate. The protein, when it is phosphorylated, has the capability to transfer that phosphoryl group to a small protein that's called HPr. That small protein, HPr, has something like 90 amino acids. Eventually that phosphorylated HPr can transfer a phosphoryl group to another protein, which has about 200 amino acids, called 2A. The feature that is very, very challenging in studying the structure or mechanism of this big enzyme I protein is that it is very flexible. There are two halves of the protein that are the amino terminal domain and the carboxy terminal domain. Eventually, it became apparent that the carboxy terminal domain is the part of the protein that interacts with phosphoenolpyruvate, and the amino terminal domain is the part of the protein that has the amino acid, which is a histidine residue that becomes phosphorylated. Histidine is at position 189. When you try to visualize the way that that enzyme I works, it is that the protein interacts with phosphoenolpyruvate at the carboxy terminal domain. In some fashion, the phosphoryl group from phosphoenolpyruvate that is bound to the carboxy terminal domain manages to try to transfer a phosphoryl group to the histidine in the amino

terminal domain. A lot of studies were directed to try to understand how that transfer of the phosphoryl group from the C terminal domain to the amino terminal domain actually occurred, and that turned out to be one of the most challenging aspects of studying the mechanism and the structure. That protein was so large, it was eventually regarded as the most challenging aspect of structure. Because we were able to break the enzyme I into the two domains, a lot of the focus of attention from the mechanism and structure was directed at the isolated amino terminal domain. The amino terminal domain cannot be phosphorylated on the active site histidine because the C terminal domain is involved with the binding of phosphoenolpyruvate. However, the amino terminal domain has the capacity to interact with the next protein called HPr. If you phosphorylated HPr, to make phosphorylated HPr, that protein has the capacity to phosphorylate the active site of histidine. It's kind of an interesting aspect of that that structure.

Somewhere along the way, I began to have an interaction with an old friend that I knew when I was in the Dental Institute [National Institute of Dental and Craniofacial Research], who had now moved to the Heart Institute [National Heart, Lung, and Blood Institute]. Her name was Ann Ginsberg, and she was a physical biochemist. At that point she had moved to Building 50, and when I was in Building 50, I had a very close association with her, and she became very interested in doing studies on the enzyme I of E. coli. The focus of attention that Ann Ginsberg made with proteins of the PTS, that I supplied to her, is that she concluded studies on ways that you could take the two isolated domains, the amino terminal domain and the carboxy terminal domain, and characterized the way the two domains interacted with each other. Ann did a lot of studies on that. At one point, there was one very interesting study she completed that showed that there were opposing effects of phosphoenolpyruvate and pyruvate on the dimerization of E. coli. We spent numerous years doing that as well.

We also had some interaction with Saul Roseman, who was the discoverer of the phosphotransferase system when he was at the University of Michigan. At that point, he had become a professor at Johns Hopkins University. Around 1985, Saul Roseman began to focus his interests on the activity of the downstream protein in the phosphotransferase system. There's a protein that was called 2A that was able to interact with the phosphorylated form of HPr and become phosphorylate. And Roseman, at that point, had shifted his interest in trying to elucidate any kind of regulatory activities of the 2A protein. When Roseman saw the report that I made that the phosphotransferase system had a connection with E. coli adenylate cyclase, he wanted to have a collaboration with me. He sent a student of his named David Saffen, to work for a while in my laboratory. The consequence of that was that we published a paper showing that the inhibition of adenylate cyclase activity by nucleotides was connected with this 2A glucose protein. That was an interesting interaction as well.

The other kind of thing that's worth mentioning is we had purified proteins in our hand, and we took advantage of an interesting technique that was called "ligand fishing." The way you did that is you could attach a purified protein to a solid support, and then once you had this solid support with a particular protein bound to it, you could then take a crude extract of E. coli that had many different proteins in it, pass it through a column with its solid support that was bound with a unique protein, and see if there was some protein factor that could be bound to the solid support because it recognized this unique protein. In one of the studies that we did with ligand fishing, we found that this 2A protein on the glucose transport system actually bound a protein. When we characterized this protein, it turned out to be a previously undescribed protein that we named a "fermentation respiration switch" protein. We characterized that. In another ligand fishing experiment we did, we bound the phosphor transferase protein HPr to it. We discovered that the HPr tightly bound to a well-known enzyme in E. coli called glycogen phosphorylase. Then we subsequently embarked on a study describing the regulation of glycogen phosphorylase by HPr. That was an interesting use of a clever ligand fishing technique.

Another thing that we did was we collaborated with a scientist whose name was Gus Wang, who was involved in various aspects of characterizing various proteins. One of the aspects that he recognized in studying this 2A protein was that it had a unique membrane anchor in the n-terminal region of the 2A glucose, which fit in with the idea that a lot of the transport function associated with the phosphotransferase system was localized to cell membrane. I think I had mentioned previously that we had also been involved in a collaboration with my friend Ron Kaback at the University of California, who spent essentially all of his career working on lactose permease. That had previously not been identified as a protein that had any influence by the phosphotransferase system that we were studying. It turned out that when we looked more closely, we found that this 2A glucose protein had the capability to bind and regulate the activity of lactose permease.

The next thing I'd like to talk about is the explorations that we did that dealt with actual structures of the PTS proteins. I've mentioned before that of the three proteins of interest of the PTS, the smallest protein was the protein called HPr. From the standpoint of elucidating three dimensional structures of proteins, at the time that we started this, getting three dimensional structures of proteins became increasingly difficult as the size of the protein increased. We were able to make some studies on proteins, and one of the side approaches that we took was that, instead of working in the E. coli system, we decided to look at structures of PTS proteins in another kind of bacteria called mycoplasma. The interesting feature of mycoplasma is it's one of the very smallest organisms that there is. Eventually it appeared that it was either a precursor or related to the evolution of gram-positive organisms, while E. coli is a gram-negative organism. One of the postdocs in my lab developed a relationship with a crystallographer who was at a structural biology laboratory associated with the University of Maryland, whose name was Osnat Herzberg, originally from Israel. We cloned the gene for this HPr protein from mycoplasma, and she deduced the three-dimensional structure of the HPr protein and provided good evidence on this basis that it was evolutionarily related to gram-positive bacteria.

We then went on to attack the three-dimensional structure of the next larger protein in the phosphotransferase system. The protein was called 2A glucose and continued collaboration with Osnat Herzberg on that three-dimensional structure. The analysis of that three-dimensional structure suggested that there was an interesting binding surface on that protein that allowed it to interact with numerous other proteins. A very interesting feature of the HPr protein from mycoplasma as well as from gram-positive organisms, was that while the HPr was phosphorylated on histidine number 15 by a phosphoryl group coming from enzyme I, there was also a site on the HPr that was at position 46, which was a serine residue. And that serine residue could actually be phosphorylated, not by phosphoenolpyruvate, but by ATP [adenosine triphosphate]. The consequence of the phosphorylation of HPr on the position 46 was that it had a regulatory role in the activity of the HPr. We also took advantage of having the gene for the HPr clone by doing a mutagenesis study that characterized the region around serine 46 that was essential for the capability of that serine 46 to be phosphorylated. It turned out that residues 48 and 49, which were right near the 46, as well as some other residues around 51 to 53, were essential for the regulation of the HPr to be phosphorylated by the kinase. It was an interesting side effect of that as well.

The next study went back to analysis of the structures of proteins associated with the PTS in E. coli. For that activity, we interacted with a crystallographer that was in the arthritis Institute [National Institute of Arthritis and Musculoskeletal and Skin Diseases]. His name was David Davies—very well known in the crystallography area. And at the same time, we developed a collaboration with a nuclear magnetic resonance spectroscopist named Marius Clore, who also worked with his wife, Angela Gronenborn. There was an overlapping interest in doing crystal structures by David Davies and solution structures by the laboratory of Clore and Gronenborn, which actually were in the same building, Building 2 of NIH. At that point in time, we had gotten structural information about HPr, the smallest protein, and 2A glucose, the next larger protein. We were approaching the challenge of trying to get structures associated with enzyme I. But because enzyme I was so difficult to manipulate because of that two-domain structure, we were concentrating mainly on the amino terminal domain

of enzyme I, which was about a molecular weight of 30,000—which at that point in time was considered to be extremely challenging. But David Davies, he was able to get this crystal structure of that amino terminal domain of enzyme I. Somewhat after that, with the Clore and Gronenborn lab, we got the solution structure of enzyme I, which basically agreed with the crystal structure.

Following up on that in the Clore and Gronenborn lab, we approached getting a structure of the complex of enzyme I amino terminal domain with the HPr protein. They got a structure of that complex. Eventually, in addition to that, we found that when the HPr complex with the 2A glucose was also deduced, the question was how you could explain the unique aspects of the structure of enzyme I complex to HPr, compared to the structure of the complex of HPr and 2A glucose? The interesting feature was that the binding surface of HPr that would interact with both enzyme I and 2A glucose was characterized as a protrusion, something that stuck out, while the binding surface on enzyme I and also on 2A glucose that bound to HPr was a depression. This protrusion on HPr fit into a depression on enzyme I, or a depression on 2A glucose. Since it was basically the same kind of a bump on HPr that could interact with enzyme I or 2A glucose, it indicated very clearly that in order for a phosphoryl group to move from enzyme I to HPr to 2A glucose, it couldn't function as one big complex. It had to be the case that when enzyme I, in a phosphorylated fashion, would move a phosphoryl group to HPr. In order for that phosphoryl group on HPr to then move to 2A glucose, there had to be a separation, a dissociation, of the complex, so that the mechanism of the phosphotransferase pathway had to be that enzyme I became phosphorylated, interacted with HPr to form a phosphoryl transfer to HPr, and then that complex broke apart to make a free form of phosphorylated HPR, which would then interact with 2A glucose. That had a very profound influence on understanding the mechanism involved with phosphoryl transfer in the phosphotransferase system.

The next thing I'd like to point out is a very interesting development in the field of phosphor transfer. It turns out that around 1984 I went to a scientific meeting in San Francisco. And while I was there, I met an Israeli scientist. His name was Jonathan Reizer. He was at that time working in the laboratory of Milton Saier at the University of California, San Diego. Milton Saier had been a postdoc of Saul Roseman, who discovered the phosphotransferase system. Milton Saier was continuing to work on aspects of the phosphotransferase system. Jonathan Reizer knew that I was working at that point on the phosphotransferase system, and he expressed great interest in coming to work in my lab. He actually then did come to my lab and spent a period from something like 1985 to 1988 working in my lab. The thing that was most interesting about Jonathan Reizer was that his wife, Aiala—they were Israelis—was an expert in informatics. They had worked together independently of the Saier lab examining the whole genome of E. coli, and they were hunting for interesting characteristics of the genome. They discovered, in searching through this E. coli genome, that there were additional sequences that were homologous to proteins of the phosphotransferase system. They found a sequence that, in addition to the enzyme I sequence, looked very similar to enzyme I, and they found another sequence that looked very similar to HPr of the phosphotransferase system. And they found another sequence that was very similar to that 2A glucose of the phosphotransferase system. Well, it turned out that the genes that encoded the analog of HPr and the analog of 2A glucose were on a unique operon that was very similar to a sequence that was involved with regulation of nitrogen metabolism. Because of that, and the fact that in searching around for some acceptor of phosphoryl group from that 2A analog, they couldn't find one. They speculated that this whole system of new genes that they found were involved with nitrogen regulation, and they named the enzyme I analog enzyme, enzyme I NTr for nitrogen. And they named the analog of HPr, NPr, which stands for nitrogen protein. And they named the analog of 2A to 2A NTr. So, then there was this family of proteins that were apparently analogous to the phosphor transferase associated with glucose transport—another whole pathway that didn't seem to be involved with transport, but with regulation, somehow, of nitrogen.

We got involved, as a result of that, in a focus of interest which probably was occupying the main part of my interest for many years after that—studying the structure and mechanism of these proteins in the pathway. We collaborated with Gus Wang, who was at that point at the University of Nebraska, in determining the structure of the 2A protein for NTr, and he got the solution structure of that. It turns out that, at about the same time, some other laboratory had gotten the crystal structure of that 2A NTr. When they reported the crystal structure, they indicated that the information there indicated that the 2A NTr was actually a dimer of the protein. Because the solution structure that was determined by Gus Wang indicated that it was a monomer, the obvious conclusion was made—that the crystal structure showed an artifactual formation of a dimeric crystal that was just an artifact of the crystallization and that did not indicate that it was really the true structure. That was useful. The NMR structure Gus Wang got for the analog of HPr, which was called NPr, indicated that it was a very similar structure to that of the HPr of the glucose pathway. However, it turns out that we found that the HPr, which we had shown would interact with and regulate the enzyme glycogen phosphorylase, did not have the capacity to interact with and regulate glycogen phosphorylase. There obviously was some difference there, and there was a different kind of a feature of NPr that was involved in nitrogen regulation. Subsequent to that, we were able to demonstrate that the unphosphorylated form of NPr played a role in regulating lipid biosynthesis. Again, and keeping with the idea that those proteins of the nitrogen pathway were not involved with transport but rather in regulation, we also subsequently showed—with a Korean scientist that was working in my lab, whose name was Yeong-Jae Seok—that the dephosphorylated form of 2A that was called 2A NTr, was able to derepress genes that were involved in the expression of branch chain amino acids, again fitting in with the idea that it was nitrogen regulation. There was another study done by Seok where the ion potassium could have an effect on the 2A NTr that indicated a kind of regulation by potassium of interaction with this factor, called sigma, that was involved in protein expression.

There was another kind of study that was done with Seok, who has by now gone back to Korea and eventually became dean at Seoul National University, where we continued a collaboration. He was able to show that the enzyme I of NTr had at the amino terminal part of the protein, a region that was called the GAF domain, and that domain seemed to be essential for a regulation of the autophosphorylation of the enzyme I that was performed in a reciprocal way by the two compounds—one, glutamine, and the other alpha ketoglutarate. That observation provided essentially good information that the phosphorylated form of enzyme I NTr had a way of sensing the availability of nitrogen.

Eventually it turned out that, in the collaboration that we had been having with Osnat Herzberg, we had this dream of being able to get a three-dimensional structure of enzyme I with both domains. Up until that point, the only success that had been made for getting structures was using the isolated amino terminal domain. She was able, eventually, by using some tricks, to get a structure of the full-length form of enzyme I from E. coli, which is a major accomplishment of getting the structure. She was able to lock the two domains, the amino terminal domain and the carboxy terminal domain, in a form that was able to be crystallized. On the basis of getting that crystal structure, she was able to formulate the model, by which the two domains had a kind of a swiveling relationship whereby the protein could exist in an open form that would interact with phosphenol pyruvate, which could then swivel to close up so that the bound phosphenol pyruvate could then get placed in a kind of symmetry that would allow a phosphenol pyruvate to then interact with the histidine 189 in the amino terminal part of the protein and transfer the phosphoryl group to histidine 189 to form the phosphorylated form of enzyme I. At which point the swivel could open up to reveal the open form again, which was now phosphorylated, which could then bind HPr to become phosphorylated HPr. That was a major accomplishment in terms of the structure of that protein. That was a very interesting development.

Well, after having these structures of the complex of enzyme I and HPr deduced, and the complex of NPr and enzyme I NTr deduced, the question arose of whether there was some kind of competition for binding of these

two proteins. When I moved from Building 36 to Building 50, I began a collaborative interaction with a crystallographer in that building, Susan Buchanan, as well as with a nuclear magnetic resonance spectroscopist, whose name was Nico Tjandra. They were both interested in having a collaboration to try to understand the significance of having in the cell these two different complexes floating around and having a potential for some kind of mistake forming, in terms of these two proteins being bound to each other. The kind of study that they were able to complete was to get the structure by crystallography with Susan Buchanan and the solution structure by Nico Tjandra of that enzyme I NTr amino terminal complexed to NPr, to make a comparison of the two different complexes—that complex and the one on the carbon pathway. They used some unique approaches to trying to get a sense of the potential competition for those two pathways. They published a paper, a joint paper, on the specificity for these two kinds of complexes to compete with each other and used some very interesting aspects of doing a comparison of the two for competition.

I would like to conclude by saying that by the time this study of these investigators in Building 50 had completed, I had become a Scientist Emeritus in the Heart Institute—since about 2006. I had been completing the stuff that I have been doing with all of these people. By the time I moved to Building 50 in 2000, my life changed because I had no more postdocs to mentor, and I had no more administrative responsibilities. Because I had left the Nirenberg laboratory to join another laboratory in the Heart Institute headed by Edward Korn, I was able to go back to a lifestyle where I could work in the lab by myself, unhindered. During that whole period of time, since about 2000 to 2016, for about 16 years, I was now back in the lab doing stuff by myself. I spent most of that time taking advantage of my capability to produce purified proteins on my own that I could then use in collaborative studies, one of whom was with a scientist named Rodney Levine. His expertise in mass spectrometry allowed us to do some innovative experiments with our purified proteins. Essentially all of my time now was devoted to making proteins, developing collaborations with structural biologists, and having these collaborative studies going on. I found that to be one of the happiest periods of my scientific career. In about 2016, I and my wife moved into a retirement community, and we started a different kind of life. At some point, Edward Korn also moved to the retirement community where we spent some time reminiscing about our careers at NIH. I kept up some contacts with people in that Building 50 community—one of whom was Rodney Levine that I keep in touch with on a regular basis to keep up with what's going on in that area of science.

That pretty much summarizes where I was for some time. I hope that this serves as the final chapter of that oral history.

Higingbotham: Yeah, thank you so much. Dr. Peterkofsky. It was very interesting hearing about your career. Thank you for sharing all of this with me. I know it was many sessions, but we wanted to get your whole career.

Peterkofsky: Okay!