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Oral History Project

Interview with Dr. Gary Felsenfeld

Conducted on September 10, 2019 by Kenneth Durr

KD: This is an interview with Dr. Gary Felsenfeld for the NIDDK Oral History Project. Today is September 10, 2019 and I'm Kenneth Durr. Dr. Felsenfeld, thanks for meeting with me today.

GF: My pleasure.

KD: I want to go back to the beginning, talk about your growing up in New York City and a little bit about your education and how you got interested in science.

GF: I probably got interested in science when I was taken to a well-known physician because I had allergies, and he allowed me to see my hand in his fluoroscope, something that wouldn't be allowed any longer. I put my hand in, it goes into between the source of the x-rays and the screen, and there were my bones, and I thought this is cool. We didn't have that expression or the equivalent. Of course, no one knew what it was to be a scientist. That wasn't exactly a profession that was well-known among people among people growing up in New York City in the 1930s. So, I thought I wanted to be a physician and my parents thought that was great, and this went on for a while.

In the beginning in my early teens, I became very interested in science and I went to Stuyvesant High School, which was special, it still is, the most famous now science specialty high school in the country. And there I met a whole bunch of other kids, boys, because it didn't have girls then, who had my own, also shared my peculiar interests. That was the beginning.

Then I was encouraged to enter the Westinghouse Science Talent Search and I won a second prize, which meant I came to Washington. And amusingly they took us, 40 of us, they took us all out here to the NIH campus, and I believe I was taken to Building 2. And we were taken to some labs on the top floor, and I said to myself, if this is what it means to be a scientist, I'm not going to do it. It was pretty grubby and of course as fate has it, that's where I ended up as a scientist when I came to NIH, at least for part of my career.

KD: Hopefully it's not so grubby today.

GF: But it's not a lab anymore. It's an administrative office.

KD: So, you must have made your decision to do undergraduate work in pre-med.

GF: Well that's exactly right. After the Westinghouse Science Talent Search—I was just thinking about this the other day—there were various people who talked to the prize winners, and a couple of them homed in on me and said, "You know, you don't have to be a doctor. You could be a scientist as a career." That was news to me because no one in my family or in any of their acquaintances really knew. I remember at one point when I was studying chemistry, a friend of the family said, "What does that mean to you? You'll run a drug store when you're finished, right?" That was it. That was not an uncommon response.

I went from Stuyvesant to Harvard and at Harvard I found a tutor named John Edsall, who became the editor of the *Journal of Biological Chemistry* and was one of the earliest people to apply physical chemistry and its way of thinking to biological problems. John took me on as a tutor and for three years I met with him once a week, essentially one on one, and we read through as many advanced books as I could read through in my spare time because I also had courses. And slowly he began to talk about himself, which is very unusual, for a Harvard professor to talk about his personal life, not done. His father had been the Dean of Harvard Medical School and his father had forced him to go to medical school, he told me. And he said, "You don't have to do that, you should go to graduate

school." My parents of course, my father said science is a hobby. It's not a full-time job and you'll never make a living.

I applied to two schools, to Cal Tech for graduate school and to Harvard Medical School, and I was accepted to both. And I went to see a very famous professor of medicine there for an interview, at Harvard Medical School, and I told him my concerns because they were going to give me a scholarship. He said, "You know, if you really want to do science—I wanted to do science and it turned out I couldn't because I'm a physician. But if you want to do science don't come here. Go to graduate school. If you change your mind in the future, we'll take you." So, I'm just wondering whether that offer is still... It might be nice to try an alternate life just to see where it would lead.

KD: That was pretty early to go for a Ph.D. There wasn't a lot of that.

GF: There wasn't a lot and there's a good reason. When I went to Cal Tech my thesis advisor was Linus Pauling, a very famous chemist. He had just been President of the American Chemical Society and given a famous speech in which he said, "Anyone who chooses a career in chemistry should take vows of poverty." And I thought that's wonderful. That sounded like a life dedicated to poverty and knowledge. Fortunately, it didn't pan out that way because just about the time I moved there, the NSF began to fund scientific research in a big way, the National Science Foundation, which was at that time a pretty small operation, started to ramp up.

My first year, I was a teaching assistant and that's how I made enough money to live. But my second year, the NSF began its pre-doctoral fellowship program. I got one of those. And I think actually Cal Tech graduate students had a large proportion of them, but there weren't that many.

KD: Is this the late '40s?

GF: No, here we're talking 1952. I graduated at Harvard in 1951. So there I was. I had been told by my tutor John Edsall that the future of biology was in application of physical methods. And Pauling was essentially a physicist, although of course he applied himself to biological problems, and I actually ended up working on the theory of ferromagnetism, the quantum mechanical theory, which has nothing to do with biology. But it's okay. It was discipline.

KD: Was that assigned?

KR: Well it was complicated. What Pauling did was he generated ideas at the rate of perhaps five a day. And his secretary would type out the suggestions for research on little slips of yellow paper, I remember, and they would go in your mailbox, and not only in your mailbox, in the mailboxes of all the faculty. And many of the faculty became famous for actually pursuing the suggestions that appeared on these little yellow slips. The advice I got from one of the senior post-docs was, "Just read them. Discard the ones that don't seem sensible. When you see one you like, do it and forget all the rest." And that's what I did. This appealed to me. I don't know why.

The fact of the matter is I can't understand my thesis now, my Ph.D. thesis. It's full of mathematics, it's full of physics and I was pretty good at it, and I actually thought for a while about making that my career, even though I was really interested in biology. And the driving force there was there weren't that many jobs. So, one of the questions was you work, just as we do now, in the marketplace; you choose a career that matches the job market. That was the way it was.

One day I went to see Richard Feynman, the physicist whom I knew because he knew all the graduate students, even though I was in chemistry, and I said, "I can't make up my mind whether this is a good thing to do, to stay in quantum chemistry." And he answered, "Well, are there any, can you foresee any simplifying assumptions that would allow you to solve the problems in a relatively straightforward way?" Of course one of Feynman's great contributions to physics was to solve complicated problems by rather remarkable

methods. I said, "No I didn't." I thought actually the way it was going was computers, because we were just beginning to see digital computers and the kinds of problems, we had in chemistry would best be solved that way. He said, "Well\, if you can't see a simplification, get out." And so I did. That's not quite true because actually what happened was then I finished in three years.

I got my Ph.D. and Pauling said, "I won't let you do a biology post-doc." I won't let you. In those days your thesis advisor was essentially in loco parentis. He said, "You have to have another year of theory, so you have to go to one of these three places."

I ended up then going for a post-doctoral year to Oxford to work with a man named Coulson, who was well known for studies of molecular structure, theory of molecular structure. Actually, there I worked on the structure of the chlorocuprate anion, which I was able to predict using the so-called crystal field theory, my last theoretical contribution. That one I do understand.

KD: Is this straight structural chemistry?

GF: Not structural chemistry. I might as well say the chlorocuprate anion is a copper 2+ with four chloride anions around it, so it has a negative charge of 2. You expect it in principle to be tetrahedral, but there were forces having to do with the splitting of the d orbitals of the copper, which causes the whole thing to flatten. When it flattens, the chloride ions repel each other and that forces it back the other way, and there's a compromise between the two forces. I calculated that and then the next year, then someone did the structure and I was right. I was very pleased with that. That was my last theoretical work.

Then what happens is before I go to Oxford my draft board calls me in because I have to get permission to go, you weren't free to travel without, once you finished your education. I remember the story well. There was a lovely gentleman and he was obviously very kind, and he said, "We're going to let you go to England for a year." He said, "But there's something we would like you to do while you're there." "Yes, sir," I

said. He said, "We'd like you to get a commission in the military because otherwise you're going into the infantry when you come home." I took that to heart.

So, while I was in England, I began to make arrangements. What actually happened was I had gotten to know Alex Rich who became a well-known scientist. He was a post-doc out of Pauling's group at the same time I was a graduate student. Alex also was in the doctor's draft, so he had to have a commission. He joined the Public Health Service. As the NIH Clinical Center just opened in Building 10, he got a lab there and he invited me when I came back, he invited me to join along with another colleague from Cal Tech by the name of David Davies, who became an eminent structural biologist. I returned from England, having solved the chlorocuprate anion ion problem, and immediately began working on problems with nucleic acids.

KD: Is this NIMH?

GF: This is now at the Mental Health Institute, that's right. A lot of interesting things are going on at NIH at that time. Childhood leukemia, Emil Freireich and his colleagues are working, I think a floor or two above or below us, I can't remember. But I do remember the elevators being full of awful sights of children and distraught parents. I think of that and the revolution that was happening to the treatment of childhood leukemia right under our noses at that time. We didn't know how exciting that was.

KD: How did you get to nucleic acid?

GF: Well mainly because Alex, Alex being a very social type, had struck up an acquaintance with Severo Ochoa. Ochoa won the Nobel Prize, but at that time I don't think he had. Because in fact what he won the Nobel Prize for was the discovery of this enzyme, as I recall, called polynucleotide phosphorylase. The name is correct. It's an enzyme that degrades RNA, but who knew in those days. He and his colleague Marianne Grunberg-Manago, who became an eminent French scientist, they drove it backwards. So they started with ADP or UDP, and drove the enzyme backwards. You ended up with

polyadenylic acid or polyuridylic acid, and Alex began to study those. And it turned out that when you mixed Poly(A) and Poly(U) you got a two-stranded structure. And Alex was doing x-ray diffraction of fibers, and David Davies did fibers of Poly(A) and Poly(U) and showed that they had the duplex structures, I recall, not the same as DNA, but it's closely related.

My job was to study the physical chemistry, so I began to do experiments. I had actually had some experimental experience as an undergraduate because I did an Honor's thesis at Harvard in John Edsall's lab and I had some lab experience. My job was to study the kinetics, how fast that these things react and exactly what was the stoichiometry, it was one to one, one strand of A and one strand of U. I did this. I measured the kinetics which were tremendously fast. Then one day I looked at the data and it didn't seem to fit one to one. I repeated the experiment and it still didn't fit one to one. It looked as though it might be two strands of U and one strand of A.

David Davies and I were in a small room together and I said to David, "Is there any way there could be a third strand that accommodated the structure?" There were no computers, but we had models and these models were these brass rods bolted together and big brass plates representing the bases. David said, "Let's go next door and look at the model." It's the sort of thing that happens very seldom. He had a Poly(A), Poly(U) duplex. He picked up an extra U and he said, "Let's see, does it fit here? No. Oh look, it fits here." And that was it. That became then the first triple stranded structure. And also, the second U was in what became known as the Hoogsteen position, except it was quite a while before we identified it in another structure, but we didn't know what to call it. It just was the third strand.

Then Alex and David, and I published this short note, I think in *Journal of the American Society*, and that's another thing to remember. There were very few places you could publish any of this stuff. There were very few journals, first of all. The term Molecular Biology was just being invented, and the *Journal of Molecular Biology* didn't exist yet. So you published either in the *Journal of Biological Chemistry*, which was a bit skeptical

about whether this was really biochemistry, or you published in the *Journal of the American Chemical Society*. That was about it. On the other hand, the advantage was you only had to spend one half day a month reading literature, that was it.

KD: Did you coin the term FDR triplex?

GF: No, that was a Russian colleague who called it FDR. That did not occur to us. We didn't call it by our names.

KD: What's the significance of that?

GF: That took a long time to figure out. It's been now a large number of years and it's actually only in the last few that it's become clear that triplex structures have important biological functions. They function in the organization of certain kinds of RNAs. And actually, a couple of years ago, maybe last year, we published a paper in which we showed that in the control of human beta globin gene expression there is actually a feedback mechanism in which the RNA that's made has a segment, the globin RNA, that forms a triplex with the DNA of the regulatory region of the globin locus. So the implication is that if you over-produce that message it will feed back on itself and slow itself down, so it's auto regulatory. There have been actually quite a number of papers now showing triplex formation as important regulatory mechanism. But you have to be patient.

KD: What was the reception to this paper if you came out with this triplex, the question is what does it mean?

GF: Nobody worried too much about that. We were in the "gee whiz" stage of molecular biology and it got a lot of attention. Then people said, we don't know what it means. You have to remember there were no ways to study function in living cells yet. All these wonderful methods we have now just didn't exist. Until we had DNA and RNA sequencing, a lot of this was very foggy.

KD: Shortly after that, you decided to go to Pittsburgh.

GF: Yes. So, I served my whatever the number of years, two or three in the Public Health Service. I had fulfilled my military obligation and it was time to go out in the world, and there weren't too many places to go. Pittsburgh had a biophysics department and that was unusual, and it seemed like it might be a place to start, so we moved there. I applied for and got an NSF grant and an NIH grant, and I remember very well that I filed my NIH application. And the person who managed my grant here at NIH phoned me up and said, "You know, you haven't applied for enough money." How quaint.

KD: What a nice privilege.

GF: In those days they were looking to expand. That wouldn't happen to anybody today, not even a Nobel Laureate.

KD: What was your area of study?

GF: That's interesting, two things. One is I became very interested already when I was still here in the whole issue of what stabilized multi-stranded structures. What were the energetics, because I was really a physical chemist and my goal was to apply physical methods to biological systems. So, I began to study the effect of ionic strength on stability and showed that you had to raise the salt to get two things to combine. You had to raise it more to get the third strand on because you were packing a lot of negative charge putting the backbones together, and you had to screen it off with more salt in solution. That was the kind of thing I began with.

At the same time, I went back to my interest in metal ions because it was early days for DNA. The structure of DNA was published in 1953, which was my second year of graduate school. Its impact was not a while to be felt. And I thought metalloproteins were a way for me to apply my quantum mechanical background. At one point here at NIH, in

the evening, I taught a course on quantum mechanics applied to biology because that's what I thought was the future.

At Pittsburgh, I had a graduate student who studied hemocyanin, which is a copper respiratory protein found in crustacean: horseshoe crabs, lobsters, squid, and we actually did chemical experiments that strongly supported the idea that you certainly don't have heme in hemocyanin. You had a pair of copper ions bound to the protein space just far enough apart that the oxygen molecule could span the distance between the two coppers. That was the model we proposed. Then years later that was confirmed by much more direct methods and that was sort of nice. I pushed that. I had one grant for that and one grant for studying the stability of nucleic acids. Not surprisingly, what happened was that the nucleic acid stuff became more and more interesting and clearly was the thing that was going to be the future of biology, so more and more time went into that.

Then after two years at Pittsburgh it became clear that the plan that had been presented to me that the department would expand and a lot of young people would be brought in, was not actually going to be executed. I was it and that wasn't going to work. Just at that point my colleague Marty Gellert, who's still down the hall, and a couple of other friends came to a meeting in Pittsburgh. Walking along after a session Marty said, "Do you still like it here?" And I said no. And he said, "We're forming a new lab at the NIH and maybe you'd like to come back and join us."

So, in 1961, the Laboratory of Molecular Biology was founded. Hans Stetten was the Scientific Director and he did something really remarkable. There was a big chunk of space and quite a number of positions available, and the reflex thought was you hire an eminent scientist at the top of his career and let him organize a big lab with his minions. There was a young guy, a friend named Gordon Tomkins who was already a section chief here, I'm not sure but perhaps not even that, who persuaded Stetten that instead he let Gordon organize a new lab with a lot of young new independent people and just let them go, and Stetten agreed to that. I came back. David Davies joined, Marty Gellert, Bruce

Ames, who was an eminent geneticist, still alive. Todd Miles was an organic chemist, now retired.

KD: Was Tomkins the Lab Chief?

GF: Tomkins was the Lab Chief and we invited Harvey Itano. Harvey Itano had been at Cal Tech with Pauling, and Harvey was the discoverer of sickle cell hemoglobin. He was the one who actually did the experiment showing that Hemoglobin-S was a separate entity with a different molecular structure. Interestingly, the Cancer Institute, to which Harvey was attached when he was in California, evinced no interest whatever because it had nothing to do with cancer. But Hans Stetten sensibly enough invited him here, so he joined us. Each of us had a relatively small amount of space, but we had three or four post-docs, something like that.

The wonderful thing about that was that the fields were so small that we all had read all of the literature, all of it. So we, I think Fridays, we had our lab meeting. In those days, every lab, every group came to these meetings and we all understood perfectly well, we took turns describing our work and we all understood each other's work. And we all had useful suggestions to make of what the others should do because we all had a grip on everything.

KD: Are these all labs at NIAMD?

GF: No, these are all labs in the Laboratory of Molecular Biology, within NIAMD, all of these small groups that had been brought together. It was an exhilarating time because people would just be able to make wonderful suggestions for your own work and you could do the same for them. And then slowly, of course, things got more complicated. The literatures got much more complicated, specialized, and gradually it became more and more difficult to make an intelligent comment about someone else's highly specialized work. But we still do.

KD: Did it remain that way through the ‘60s?

GF: Oh yes. And even now when we all get together and someone presents something, people, because we have so much experience, so many years of experience.

To come back to the issue of what I started to do. I continued to work for a while on stabilization of nucleic acid structures through modulating the electrostatic forces. Then I started to use, to stabilize nucleic acids, I started to use larger and larger positively charged molecules. I started with sodium ion then went to magnesium, then I went to organic charged molecules, so-called spermine and spermidine. And then I thought why not work with peptides, so I started to work with polylysine, which is a positive, all the lysines are positively charged, that’s a big molecule, and sort of beginning to be like the proteins that are found in the nucleus, which are very lysine and arginine rich, those being positively charged amino acids. So I started to use those.

KD: You’re still looking to stabilize the multi-stranded—?

GF: Yes, now looking at two-stranded DNA mostly and beginning to study the interactions of whether there is a preferential interaction of one over the other, and we published a number of papers like that. Then one day, because at the ends of these papers I began to write, and this is relevant to the study of the histones, which are the positively charged proteins found in the nucleus, which are rich in lysines and arginines. And one day as I wrote this, at the bottom of the paper I thought this is ridiculous. Why not actually work with these proteins. So, we switched, and we began to work with the proteins. And from there very quickly we began to work with chromatin, which is the naturally occurring complex of DNA and proteins, including the positively charged packaging proteins called histones.

KD: It looked like you were working on histones for a little while before you went to the chromatin.

GF: Yes. The individual histones, everyone thought they might be... But even then, I began to think I'm not really as interested in the structural aspects, the physical aspects. Really, I'd like to know how this works and I'd like to know how the chromatin helps specify which genes are active and which genes are inactive in the nucleus. That would be about the time that Howard Cedar came to the lab and Richard Axel.

KD: Mid '70s, something like that?

GF: That's probably about right. They were electrifying. Axel in particular was full of ideas and Cedar as well. Howie and I, particularly Cedar and I, began studying chromatin as a template for RNA polymerase and to show that it could be transcribed. And I became interested in how much of the DNA was accessible to transcription factors and to RNA polymerase and that became the general direction in which the lab began to go. I'm just thinking about Richard Axel in particular, who was a remarkable force in the laboratory.

KD: In what way?

GF: I'll tell this story. We had a program for MDs who were trying, doctors' draft still existed, we had a program where we offered post-doctoral fellowships in our institute. And the candidates who were successful came, and they went around from lab to lab interviewing. And one day I got a phone call from Chris Anfinsen, who is a Nobel Laureate, who was in our institute at that time. And Chris said, "We had this guy come into interview and we told him what we were doing. And you know what he said? He said you're doing the wrong thing. This is what you should be doing." Chris said, "You know, we don't like wise asses over here, but I know you do, so I'm sending him over." A little while later, in comes Richard. He sits down and he says, "So what are you doing." And I tell him. He says, "You know, you're doing the wrong thing." And I said, "I think you're right." And that's how Richard came to work in the lab.

KD: What were you doing wrong and what did he help you do right?

GF: As I recall, he wanted us to work more directly on real templates rather than artificial systems. He was one of the forces that persuaded me.

KD: What's the difference between the template and the artificial system?

GF: Well you purify the DNA and then you add histones. That's one way to do it. That's the way a physical chemist does it. The other way is you take a nucleus and you open it up or you don't. And you deal with all the mess.

KD: That's what a microbiologist would do it?

GF: Yes, that's what biologist do and that's the common way to do things nowadays. Synthetic systems have their limits. In fact, that was one of the problems. For example, with the histones, I don't think we had that problem that other people, namely people studied the individual histones and many of them had structures that you could form, that you could solve by x-ray diffraction, and they actually have no relationship—nature doesn't know about those. And that's the kind of error that can happen if you do the reductionist sort of thing, so we now commonly do things with a whole cell. It's messier, but you avoid those kinds of errors.

Then the next step was to find a really good experimental system. Fortunately, there was one. One of the problems in trying to isolate chromatin is if you try to isolate it from most kinds of cells, most nuclei are full of proteolytic enzymes and nucleases. So, both the protein and the DNA, you have to work very fast. In the early days I would have, like many other people were working with calf thymus chromatin. The way you work with calf thymus chromatin is you go up to Baltimore with a bucket full of dry ice and you stand there while they slaughter the calf and take out the thymus, which is sweetbreads. It's very good, delicious. And you immediately smother it in dry ice. You race back to the lab here and then you spend the rest of the day isolating the chromatin and doing your experiments because the next day it's all gone, and that's because the thymus is full of enzymes that destroy them.

Hal Weintraub who unfortunately died of a brain tumor quite young, Hal Weintraub and Mark Groudine, who is still active out at Fred Hutchinson in Seattle, they started to work with a chromatin from chicken erythrocytes. Chicken red cells, bird red blood cells in general keep their nuclei. We don't. So, in the last stages of erythrocyte maturation it spits out the nucleus, but birds and reptiles don't, and chicken blood is easy to get. So, we began to work with chicken blood and chicken erythrocytes, very easy to get nuclei from their blood. In fact, as a demonstration: When our kids were growing up, I used to bring them in on a Saturday and we would make nuclei from chicken red blood cells, they would take them to show and tell.

KD: I'll bet they were the only ones in class with that kind of show and tell.

GF: I'm not sure.

KD: Bethesda?

GF: Bethesda-Chevy Chase High School.

KD: So, you're able to do your experiments at a more leisurely pace?

GF: You would keep the erythrocytes in the refrigerator. You would keep the nuclei in the refrigerator overnight, probably even longer than they need, but there's no need to. The chromatin that you made was stable and we began to study the regulation of beta globin gene expression. And now things begin to get less fuzzy, I would say.

We identified regulatory sites and enhancers in the early days when an enhancer was not a very well-defined thing. We identified an enhancer that was very important for red cell, for globin DNA expression. I'm trying to remember the chronology of this, because now, given that I see it as a whole picture, it's a little hard to remember the order in which things happened.

KD: My impression was that you discovered the regulatory region when you were still working with the histones.

GF: Yes. There is an important thing. What we noticed was that there was a DNA sequence in what would have been the promoter, what we thought of at least upstream of the beta globin locus. You have several beta globin genes that are developmentally regulated in the egg, in the early embryo, and then in a mature chicken you get a different globin gene expressed. There was one transcription factor, or at least a motif that seemed to be shared not only by the globin genes, but by other genes associated with the erythroid gene, the general pattern of expression. Then we decided to look for this protein. Ultimately, we purified it, someone named Todd Evans, who is a professor at Cornell Medical School now, and we gave it the name, originally, we gave it the name ERY-F1, erythroid factor-1.

At the same time, Stu Orkin at Harvard purified it from a mouse, and he called it GF1. Someone joked that he would never stick with that name because those were my initials. There was a meeting, there's something called the Hemoglobin Switching Meeting, which was big. Every other year we met and there was a mini conference of the interested parties.

KD: How many?

GF: For this meeting, the Switching meeting has about a hundred, but there were about six of us in this mini conference. They were the interested people. What we agreed, at that time, it was becoming clear that this was the first of a whole family of proteins and that they regulated not only erythroid expression, but one was involved in cardiac development and a lot of different things. It was decided to name it after the central motif, which is G-A-T-A, those are the four bases. We call them the GATA proteins. Our protein was called GATA 1. GATA 1 turns out to be essential, not just for globin gene expression, but for a whole erythroid gene family. The other GATA proteins are equally important for many different functions.

KD: Did you develop any of the other ones?

GF: Not in the least because my focus was still on the issue of regulation of the globin genes and the chromatin structure changes that were associated with it. There are a lot of papers in there. I would have to look at the bibliography to remember.

What stands out next, we began to characterize the histone modifications, and we were one of the earliest labs to do a rather thorough study, the *Science* paper we published on the modification patterns during development. At the same time, we became interested in sort of a puzzle, namely, one of the next things was we began to look on either side of the beta globin locus and we discovered that there was a big chunk of heterochromatin upstream of the beta globin locus. Then beyond that there was another active gene, erythroid specific active gene. So, the question was, it was known that the heterochromatin liked to spread, and that would silence everything. How come it didn't encroach on the globin locus? So, we began to think in terms of there being some kind of boundary. When we looked carefully, we could see that just between this big chunk of heterochromatin and the globin genes there was a segment of DNA that seemed to be binding multiple proteins.

So we began to look at those proteins and we characterized them one by one. And one, in particular, seemed interesting because, as I recall, that one alone was sufficient in our assays. We set up an assay to test: If you had an enhancer, which is an activating element here, and the reporter gene here, typically that would be enough to turn that gene on, but we would then put a binding site for a putative blocker here and see if that prevented the enhancer from activating the promotor. In fact, we found that this one DNA sequence from that big block of stuff between the condensed chromatin and the globin locus, one segment of DNA binding one protein was effective in that assay, so we decided to purify that protein. That took a while. Eventually we did and that protein was a known protein called CTCF. What we learned about it was it was some kind of transcription factor and it had all kinds of funny properties. But what we then showed was that it was necessary and sufficient to prevent an enhancer from activating a promoter.

Then what happened was interesting. There was and still is a great scientist by the name of Shirley Tilghman. She was running a lab at Princeton at the time. She ultimately became President of Princeton, which I've always considered a great loss, but she did a great job at that as well. Then she had been studying for years, she was one of the two or three labs in the world studying something called imprinting. Imprinting is a phenomenon in which there are, of course, two copies of genes one on each of the chromosome pairs. An imprinted gene is one which is only expressed from one or the other of the two copies and is transmitted either through the female or the male.

In the case of this locus that she was studying, called the IGF-2 locus. IGF-2 is insulin-like growth factored 2, it's quite close to the insulin gene. In that case, IGF-2 is off on the chromosome copy that comes from the female and it's on, on the paternal chromosome. The question was, how did that happen. Shirley came to me at some point when she knew we were working on this enhancer blocking phenomenon, which is called insulation. She said, "I wonder," this is before we had actually discovered CTCF, she said, "I'll bet there's an insulator and that's how it's working." That really focused our attention on that area. I think we had just discovered CTCF, identified it, and she published the sequence of this region called the imprinted control region, which was the regulatory region which she had shown was determining and deciding which allele was expressed.

I remember I took the sequence and I looked for CTCF binding site, and I found several. So we immediately began to look and confirm that, first of all, that CTCF bound to the maternal allele and that would mean that the maternal allele would be shut down because the enhancer was here on one side, the gene was on the other, and the CTCF sat between them. So, what had been shown was that on the paternal allele the DNA is methylated. The DNA is methylated. Methylation occurs at a "C" next to "G." That's the classic place where DNA gets methylated, not the only, but the predominate place. Indeed, what we showed was if you methylated the DNA, CTCF no longer bound, so you no longer had an insulator, so then the enhancer could act on it. It's too simple by half. And of course, nature

never leaves well enough alone, so it's been more complex than that. But that's essentially the correct story.

We then shared this information with Shirley because we would never have done this without her. Then they did it in a mouse, then we did it in a human and we published it together in *Nature*, and that was very satisfying.

KD: CTCF was precisely the thing she was looking for?

GF: It was. She didn't know. Yes, that's exactly right. She guessed that there was an insulator there, but very little was known. There was no vertebrate insulator known. There was one in flies called suppressor of Hairy-wing and maybe one other in Drosophila. But no one had ever found such an insulating element in vertebrates. That's a while ago now, early 2000s. But CTCF has become a growth industry, I would guess, because it's turned out that it's not only doing that simple-minded thing, but it's also helping to organize, and we didn't do this, a large-scale organization of the genome.

So, it's been shown that the genome in eukaryotes is organized into big loops which are maybe a megabase in size. Then these loops are closed off by a pair of CTCFs and there are accessory factors that are actually causing the stabilization of the loop, so the CTCF is actually stopping the passage of cohesin. Cohesin is a complex that can link DNA strands together. The strands at the base are linked together, so you get big-looped domains. Regulatory elements in one loop domain find it hard to talk to genes in a separate domain, but they like to talk to ones that are in the same loop.

KD: Did you do some of this looping?

GF: No, we did not, not then anyway. That's all work in a multitude of laboratories and it's now separate. We used the tools that have been developed a lot now. That was essentially other groups.

KD: Were you Lab Chief at this point?

GF: LMB history. We have to back in time. Gordon Tomkins was Lab Chief for about ten years, something like that. He was charismatic and very funny, and had many, many job offers. In fact, at one point he thought all of us would move to Yale to form a new biochemistry department there. And we all would have gone, except he decided against it at the end, so we all stayed. Then after years of being romanced by all the major schools, actually appointed Professor at Harvard Medical School, as I recall, and turning that down, he finally took a job at UCSF. San Francisco was expanding then. They had essentially a fresh department and Gordon was finally recruited there. The fact is Gordon was a terrible administrator and we did all the administrative work. So, when Gordon left, we asked that we be allowed to run it the way we had been used to running it, which was as a commune and unusually probably the only lab at NIH. We had a rotating lab chief, each of us served for a year. We met once a month and we talked to each other. We always agreed with each other.

KD: How many lab chiefs?

GF: These were the section chiefs. About six I would say: Marty Gellert, David Davies, Todd Miles, Bruce Ames, me. I'm missing somebody. And that went on for a number of years very pleasantly. Then Allan Spiegel became Scientific Director, and Allan was right, it was probably very intimidating because when we wanted something we showed up, all of us in the Scientific Director's office and that was difficult. He said we had to pick somebody to represent the group and that was me for ten years or so. It actually didn't change a thing because we met once a month. We talked over what we wanted to do, and we agreed. We always seemed to agree and then I just went and told him what we had agreed on.

I want to talk about the lab. It was a phenomenal place. Over the years we began to accumulate members of the National Academy of Sciences. In those days NIH had only a tiny handful. We began to just get them, not just staff members, but then slowly people

who had been passed through the laboratory. I think we're up in the 20s now for present and past lab, LMB alumni who were, are or were when they were alive, National Academy members. That's without actually trying.

KD: You say it's without trying. What do you think the cause was?

GF: We attracted tremendously smart, independent people and it was place people liked to work. I think it was because we started out with a tremendous group of people. I'm thinking of the original people, Harvey Itano, Bruce Ames, David Davies, Marty Gellert and me, we all became Academy members. That's not usual, not because we helped each other to get in, because we were in different fields. It was just very high-powered science.

KD: Do you think there was more opportunity because of the way the lab was initially organized?

GF: Absolutely. I think it's the environment, a very supportive environment. In the outside world—I was offered a professorship at Harvard Medical School and I could see that there, things are different. People didn't speak to one another. They didn't want their students, their post-docs to speak to post-docs in other groups, and that's no fun. This is fun. And also, it means that a post-doc who comes to your lab is going to benefit from being able talk to all the other people in all the other labs. And NIH is altogether a culture of sharing ideas, equipment, techniques, which is not the way it is in most universities, not at all. That's why people who are here mostly stay. But Bruce Ames didn't stay. Bruce moved to Berkeley and became professor at the University of California. He's retired now. He's emeritus, but I think he's still active.

KD: David Davies stayed.

GF: David Davies stayed. He died a couple of years ago. Harvey Itano left. He went to San Diego, to La Jolla to become a professor there in the medical school. He continued to study hemoglobinopathies of various kinds.

KD: Any other LMB politics I should know about?

GF: I can't really think of any. There weren't any. We were relatively peaceful. There was no infighting. It's one of the wonderful things about NIH because you're not really competing for resources in the way you are in the universities. People will share with other people. The other thing is that there's nothing to do all day long but talk science. You come in, you're not going to have a lot of committee meetings, no faculty meetings. We meet once a month for an hour to discuss lab issues and then if we're recruiting someone then of course we have a little bit more work to do, but it's trivial compared to the kinds of obligations you have out in the university system. It's fun to come to work every day.

KD: But some of the structural things do make a difference. Let's go back to science just a little bit and talk about how you got involved in pancreatic cells, because that's what you're doing now.

GF: That's right. I guess we hadn't talked about that. When you asked me about long range interactions and loop formations and so on, I did say that not until relatively recently had we been doing that. Someone came to the lab, I can't really remember. It's because of IGF-2. Insulin-like growth factor 2 is not far from insulin itself and there is a story associated with that. When we did the IGF-2 H19 locus and the imprinted control region stuff, I went to see Allan Spiegel to tell him this news, which was significant. I said, "I would like you to note that the word 'insulin' is in there and this is the Diabetes Institute." Allan said, "I don't want you to ever think that you have some obligation to work on something related to diabetes. You do basic research and you can do whatever you please, but you don't have to." I thought that was great, which of course only egged me on. So as I said IGF2 is close to insulin, as these things go.

People had begun to think about interactions between distant elements. We didn't have the so-called high C methods that dominate technology now days. Someone came to the lab

who was interested, name was Zhixiong Xu, and just at that moment the extramural part of our institute began to support islet preparation, pancreatic islet preparation. The reason for that was there was a big move to do islet transplants in patients. Patients with Type 1 diabetes who had malfunctioning pancreatic islets, the thought was that you would give them transplants and that was quite popular. NIDDK Intramural actually got a specialist to work on that. I think it's not so popular now because it turns out the immunosuppression problems are just as serious as they are for any other organ transplant. So the cure had problems of its own.

In any case, it developed a whole series of centers that did organ preparation from deceased donors and they began to produce islet preps and you could sign up and we did sign up. That meant that we could get supplies of islets, not on demand, but whenever a donor appeared.

So we began to look at the insulin gene itself. And just at that moment there had begun to appear methods for measuring contacts within the nucleus between a given site and at another specified site to see whether there was any kind of physical contact inside the nucleus. That was called 3-C and then was upgraded to something called 4-C in which you could choose your anchor and then ask where else, anywhere else, in the genome did that anchor make contact.

It was very early days, there was no computing expertise. For analyzing the data we teamed up on Keji Zhao's lab at the Heart Institute, and they wrote the software to do the analysis. And we began to take islet shipments and to measure contacts between insulin and other nearby sites on Chromosome 11, which is where insulin is in humans. What we discovered was there were a lot of physical contacts. Then as an example we chose one, which seemed to be particularly significant. It was a gene called synaptotagmin 8, SYT8. What we showed was that the contact, actually when you fed the islets glucose, the contacts strengthened. In this particular case the contacts were stabilized by CTCF and SYT8, turned out, no one knew what it did. We showed that it actually affected insulin secretion. So now we had a kind of complicated feedback mechanism in which the level

of insulin expression affected the expression of an auxiliary protein, which would help, so when insulin goes up, SYT8 goes up and now you secrete insulin more effectively. That was a kind of physical feedback system and it was the beginning of what we then did. There weren't many examples at that point of that kind of physical contact as a regulatory thing.

KD: What was the next step?

GF: The next step actually was the technology improved tremendously and the software improved tremendously, and the post-doc is still here , named Xing Jian, he extended that. Then we switched away from islets. First of all, you really do depend on availability and every batch is different in terms of how good the cells are, how healthy. A beta cell line became available, which made human insulin. So, we studied that, and Xing was able to measure the contacts between insulin and all the other chromosomes in the genome, and there were lots. What turned out, which was amazing, is that a large fraction of those contacts are to sites that contain Type 1 or Type 2 diabetes susceptibility loci.

KD: Susceptibility what?

GF: Loci. A susceptibility locus is a region of the genome that contains a DNA sequence that's statistically associated with some trait, or susceptibility or resistance to disease, in this case diabetes. Actually in between using the islets we actually had found still another gene called ANO-1, which nobody had ever paid attention to, which we were able to show was mildly connected with a glucose intolerance in mice that had the gene knocked out. So this becomes a way to connect the physical genome to the regulatory genome. The analysis in the cell line also enabled us as an example to identify a gene previously unrecognized as having something to do, in this case with insulin secretion. It's a way to think about the problem. We're not going to try to do a comprehensive study. It's just proof of principle, if you like, and it's not just proof of principle about how insulin regulates its environment, but it's certainly the way a lot of genes are going to operate.

It's interesting, insulin and globin are examples of two genes that dominate the cells in which they are expressed. So, a beta cell, one of the major products is insulin. So you wouldn't be surprised that a lot of its strategies are devoted to seeing that insulin is made in just the right amount, and the same is true for erythroid cells or red blood cells, namely you know that beta globin, first of all, has to be matched to alpha globin. The two chains have to be made equal amounts. But the cell is going to devote a lot its mechanism to some kind of maintenance of a stable productive system.

They may be special in that way, but the whole idea of physical contact, we certainly are not the only people who thought of this. Peter Fraser, some years earlier actually had proposed so-called transcription factories in which lots of RNA polymerases are clustered together and lots of transcription factors that are going to be shared by multiple genes are there, and all those genes clustered together are centrally located to share in those factors, which is an efficient way to do things.

KD: Are you still publishing this work in this line of inquiry?

GF: Dr. Jian has been looking at issues having to do with long range interactions in the insulin locus that are imprinted. So IGF-2 is imprinted at a distance away from insulin and there are lots of questions about imprinting on the other side of the insulin gene. He's just finishing a study on that and we're just writing it up.

KD: Is this your section that's doing this particular work?

GF: This is my section.

KD: Moving up to the lab, it was interesting, the idea you were told you don't have to work on diabetes. The Laboratory of Molecular Biology can work on whatever it wants to work on.

GF: Yes.

KD: Where do you see that going? Given the past, and you were there in the beginning, where do you see the lab going in the future?

GF: I think science has changed. The kind of science we did, there still a place for it, which is people receiving problems that are really not solved, and messy problems, and clearing the shrubbery away to see the real problem, and then solving it. But one has only to look in a journal like *Cell* to see that the emphasis now, first of all, is very clinical. And secondly, in part because of that, involves massive numbers of people. Not too many papers have three authors anymore. I don't know what the average number is. But in journals like *Cell* and *Nature*, it's typically a small army. And that's rather amusing because in the history of quantitative biology, when I was starting out, we had a big influx of physicists.

They came in for a couple of reasons. One is because they were under the misapprehension that the laws of physics—there would be laws of biology that would be just as simple. The laws of physics aren't simple anymore either, but they thought it would be simpler. But mainly they came because physics had become big science. And if you read the descriptions of how these large-scale studies were where 300 people arrived at the end of the study in a big auditorium where they're told what they found, which they didn't know, and then they all go home. So, they thought, correctly, that biology was something that would allow for individual expression. And that's less true now.

I don't know what it's like to be a member of a 50-person group. I'm sure people get satisfaction from it, but it's a different personality, I would say. The group leader still, if he can actually remember the names of all 50 people, must enjoy it and I know people who do and they're wonderful.

The science is fantastic. I don't want to impugn the science. Science is wonderful. There's so much of it that nobody can keep it in mind. And I spend a lot of my time now

doing science, we do our own experiments, but we can support them with millions of other experiments that have already been done. So, you can ask all kinds of questions about whether two transcription factors are bound next each other, near each other. That's one of things I'm quite interested in, CTCF co-factors. And all that's in there, but nobody knows about it because the data was deposited in experiments and studies that were addressing other issues but buried in there are also the answers to thousands of unasked questions. That's fun, but why shouldn't it be different.

I do this experiment of thinking about an equal number of years. If you think back 50 years from founding the Laboratory of Molecular Biology, 1911, and we didn't know anything about protein structure. In fact, we didn't know anything about protein structure until Linus Pauling and that was when I was a senior in college. Up until then there were all kinds of amusing proposals for what a polypeptide looked that, none of them remotely right.

KD: So the lab took off shortly after that revolution.

GF: As did many. No question, the discovery of the structure of DNA and the structure of proteins changed the way biology was done, no question.

KD: Is there anything else that we should talk about that we haven't touched on today?

GF: I think talking about the future of science, if I were starting over, I think I would do neurobiology because there's still a chance there, at least if you have good hands, to do your own experiments. For me, I worked at the bench until fairly recently and that was the greatest pleasure. To focus on an experiment and to shut out everything else is one of the great pleasures. Now there are a few places where that's appropriate in a way. Everything is essentially pre-done for you. When we did our own experiments, we made our own buffers first, which would horrify anyone now. If I went down into a lab and said, "make a buffer," I think they probably would be puzzled. But it was always fun. If it's not fun, you shouldn't do it.

KD: It's been a lot of fun for me and I've learned a lot. Thank you very much.

GF: You're welcome.