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Interview by Ute Deichmann with Gary Felsenfeld

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In 1961 Gary Felsenfeld was appointed head of the Section on Physical Chemistry of the Laboratory of Molecular Biology of the National Institute of Diabetes and Digestive and Kidney Diseases, part of the National Institutes of Health in Bethesda, Maryland. His work started with the investigation of the interaction of regulatory proteins with DNA, and then focused on the relationship between chromatin structure and gene expression in eukaryotes.

1. From physical chemistry to biophysics

UD: You studied biology and physical chemistry. What made you choose this unusual combination?

GF: It wasn't so unusual when I did it. It was unusual to study anything. I was interested in biology – that was my main interest. As an undergraduate at Harvard, I had a tutor who was in biochemistry, biochemical sciences. His name was John Edsall.

UD: John Edsall? He was famous for his contributions to the physical chemistry of proteins.

GF: Yes, he was editor of the Journal of Biological Chemistry in his later years, but when I knew him, he was professor of – I don't know what the name was in those years – biophysical chemistry or physical biochemistry or something like that. He applied physical methods to biological problems, particularly the properties of proteins in solution. I was interested in the biology and I had fairly strong quantitative inclinations. And he said the biology of the future will involve physical

chemistry. The term “molecular biology” did not yet exist. The idea would have been thought ridiculous. So I did an undergraduate thesis with him, and in my last year when we discussed what I should do next, he said that I should go out to Caltech, which was the center for the study of this kind of biology – to the extent that there was. There was crystallography and there was a strong emphasis on physical chemistry, so that’s what I did. But Edsall was probably the first biochemist to study quantum mechanics. He told me – and he’s written this as well – that he first learned about Schrödinger’s formulation of quantum mechanics from J. Robert Oppenheimer.

UD: Quantum mechanics?

GF: Yes, he learned about it from Oppenheimer. He and Oppenheimer and another man named Jeffries Wyman, who was one of the few other well-known biophysical chemists actually working at that time, went walking in the hills, somewhere in Corsica in 1926. And he was hearing about the new and perhaps simpler way of thinking about quantum mechanics that Schrödinger had just published. Edsall was probably the very first biologist to teach himself quantum mechanics - and under his tutelage, I started to study quantum mechanics when I was still an undergraduate. We read a book on the subject by Eyring, Walter and Kimball together. I read it and each week we met, just the two of us, and I told him what I had read. That’s how I ended up at Caltech, ultimately working with Linus Pauling who was my thesis advisor. In this period, that is 1951, Linus Pauling appears on the East Coast. We all go over to MIT to hear him, and he tells us about the alpha helix. You can’t imagine how amazing that was. My last year in college I was taking a course in physical biochemistry from Edsall and others at the medical school. The structure of proteins was guessed at. And they taught us something called the cyclol structure proposed by Dorothy Wrinch. We had to learn that, but of course, it was totally wrong.

UD: She was a mathematician.

GF: The thing is, what did they have to go on? You know that the reason that Pauling came upon the alpha-helix, and others missed it, is because they did not appreciate that all the atoms around a peptide bond have to lie on a plane. Pauling knew that two ways. He knew it, first, because his understanding of valence bond theory and the behavior of chemical bonds allowed him to predict that this would have to be the case. Secondly, and more importantly, in pursuit of that he had arranged for Corey and others who were associated with him to solve the crystal structures of di-peptides and things like that, which proved that the peptide bonds were co-planar. And therefore, when he built his models, he was left with a very small subset of the otherwise rather long list of possible structures of a polypeptide.

UD: The understanding of quantum mechanics helped him understand the co-planarity?

GF: Yes. One of the things I remember - when I was in high school, I was already trying to study some biochemistry and I bought a book by a guy named Harrow. It was really the only decent biochemistry book. But I remember specifically the part of the text where they showed chemical structures like purines and pyrimidines, and instead of showing benzenoid rings, which would be symmetric, they just drew them as sort of squares and rectangles. There was no sense of the three-dimensionality of this thing. No sense that the three-dimensional structure governed the properties. That was Pauling's great contribution, I think.

I went to Caltech not long after that talk.

UD: Which year was that?

GF: I was at Caltech from 1951 to 1954. That's all it took to get a Ph.D. in those days. My thesis was on the theory of ferromagnetism. It had certainly nothing to do with biology—nothing at all.

UD: And also nothing to do with Pauling's work?

GF: Oh yes! He had a theory. And I elaborated the theory. I could tell you, I have my thesis here somewhere.

UD: Was Pauling's theory right?

GF: Probably, sort of right. But much too simple by the standards of present-day sophisticated theories.

I thought this was going to be a preparation for a career in biology because the biology of the future would make use of these kinds of techniques. So then at some point I began to wonder whether I could make a career in quantum mechanics. And there was a wonderful moment when I went to see Richard Feynman, whom I knew because Caltech was really small. The principle there (or it seemed to me) was that the students and the faculty were one big family. Feynman used to go to our graduate student parties and play the bongo drums on the back of a coffee can, actually. Anyway, I went to see him and he said, "well, do you see any simplification in the field of quantum chemistry– anything new that would simplify this complicated subject?" I said that I didn't think so. Then he said, "Well then, get out." Which is what I wanted to hear anyway.

Caltech had this arrangement where you were required to have a minor. My principle subject was theoretical physical chemistry. But my secondary field was biology. So I had to take comparative anatomy and things like that, which I liked. Then I had to take an oral exam in biology. That's when I met A. H. Sturtevant who was the chairman of my exam committee. A lovely, warm, friendly, and unpretentious person.

As an aside, I just put the finishing changes on the manuscript for the volume that you are editing. One of the comments I received was that I had attributed position effect variegation to Muller. I imagine it was Eric Davidson who said that it wasn't Muller, it was actually Sturtevant five years before.

UD: I wrote it to you also.

GF: Oh, it was you! Thank you. I have to tell you that – so I had written something earlier for Cold Spring Harbor just on the early history, really. And I had consulted a lot of people about that issue – who should be considered as the first person to recognize an epigenetic mechanism – and no one mentioned Sturtevant as the person who made this discovery of position effect variegation. I felt bad having missed that. That he didn't get a Nobel Prize is an absolute disgrace. He was the intellectual father of a lot of modern genetics.

So this wonderful oral exam consisted largely of taxonomy. There were various specimens presented to me, some in glass containers, depending on what they were. My job was to say class, order, family, genus, and if possible, species. Although I grew up in Manhattan and I'd never laid eyes on any of these animals, I had studied very well, and I did define things entirely from my memory of the official descriptions. So all went well until the last, when I was presented with a jar. It appeared to contain some sort of insect-like thing but with many legs. I said, "Centipede." (Naturally, I had never seen a centipede.) And, of course, that was his joke. It was a bunch of insects all jammed together into one jar. That was the sort of thing; that was his spirit. Anyway, that was the end of my biology studies.

Then, of course, in 1953, comes the DNA structure. And what happens? Crick comes to Caltech; there is a big meeting on proteins that Pauling convenes. And I get to meet Crick, and then Watson comes and a whole bunch of other people.

UD: Crick came to a protein meeting?

GF: Yes, because one of Crick's major contributions before '53 concerned the X-ray diffraction pattern of a protein coiled coil. So now comes the DNA structure. Before it is published, early '53, there is a big lecture: Pauling announces that he's going to describe the structure of DNA.

UD: Poor Pauling.

GF: Yes, poor Pauling, although recall that he is already very famous for his other discoveries. And so I go along to it, of course. You wouldn't miss it. But here I am, I've been reading Harrow, I've been working on ferromagnetism. I have no idea why DNA should be important. It wasn't discussed much in my courses. They talked about thymus nucleic acid and yeast nucleic acid - one was DNA and the other RNA. And of course, coming out of the dark ages of polymer chemistry in the early 1930s, before then, polymers were all thought to be loose aggregates of small molecules -

UD: Oh, you mean colloidal theory.

GF: Yes. And then, of course, you have all the work that proves that, in fact, proteins are not colloids made up of small sub-units. But I remind you, you have Svedberg, you have Staudinger, and that changes everything. But in fact, for quite a long time, the nucleic acids were thought to be aggregates of fundamental sub-units consisting of the four different nucleotides. And, in fact, the recipe for studying RNA at one point was to boil it in sodium hydroxide to disrupt the non-covalent bonds and get the essential tetrad of four nucleotides – of course that's how you degrade RNA: you hydrolyze the bonds. And similar ideas existed about DNA. But if your mindset is that it's not a high molecular weight molecule, then it clearly cannot be the genetic material. It takes all of the new polymer chemistry to percolate, before anyone could even think that DNA is a high molecular-weight substance that might have some biological significance.

So that's what's going on before, and I don't know at what point people began to realize that DNA was really a macromolecule, but certainly in the early 1950s they did know.

UD: They knew from the late 1930s, but they still believed that it was a regularly built molecule without major biological significance. Then came the experiment of Avery.

GF: The experiment of Avery, Macleod and McCarty showed the biology.

UD: Afterwards Chargaff showed the species specificity of different DNA molecules, and that paved the way for everything that came afterwards.

GF: And he did enunciate his 'rule' that A=T and G=C, very important for Watson and Crick, even though Chargaff didn't seem to appreciate what they did with it.

So time goes on and I've now finished my second year, beginning my third year, and it's time to think about where I'm going to go for my post-doc. I wanted now to turn to biology.

I should go back a bit, because there was an important thing that happened between graduating Harvard and going to Caltech in the fall of 1951: Edsall arranged for me to have a summer at Woods Hole working with the man who had been his mentor when he was an undergraduate at Harvard. His name was Alfred Redfield. He was a famous biologist who worked on respiratory proteins, particularly hemocyanin, which is an arthropod and mollusk respiratory pigment; it's a copper protein with a beautiful blue color. At that point Redfield was the scientific director of the Woods Hole Oceanographic Institution. So I spent the summer in his lab, and that was very important because it actually became a direction I went in for a while - to study metalloproteins. I'll come back to that in a bit.

Anyway, I told Pauling I wanted to go work with Linderstrom-Lang who was the great protein chemist of his age. He was in Copenhagen. And Pauling said, "No, I won't approve of you going there." In those days - you know. Nowadays, someone finishes graduate studies, goes wherever they can get in, and wherever they please. But when my mentor said, "I won't approve you to go there," that was just like – that was the end; you did whatever you were told. He said, "I think you need another year or two of training in quantum mechanics, even though you want to do biology. It will be good for

you." And he told me where I had to go. I had a couple of choices, and one of them was to work with Charles Coulson, who was Rouse Ball Professor of Mathematics at Oxford. And who was one of the premier investigators applying quantum mechanics to molecular structure. So I went there.

And by the way, all this time at Caltech and then afterward with Coulson, my roommate was Martin Karplus. He's a friend since college. But in any case, a lot of people were at Coulson's at that time very well-known people; one of them Bill Lipscomb who won the Nobel Prize for his work as a structural chemist.

UD: Karplus received the Nobel Prize right now [2013], right?

GF: Yes. The non-Israeli member of the group of three winners.

UD: So you went to Coulson, and…

GF: I went for a year, and there I did a very nice thing, which was to study the structure of the chlorocuprate anion. I don't think you want to know more about it than that.

UD: No.

GF: Or maybe you do. It's a cuprate ion with four chlorines attached. And its crystal structure had just recently been solved; it was a flattened tetrahedron. I was actually able to predict its conformation quite accurately, it turned out. It's probably just luck. But it's the last thing I did in quantum chemistry.

Then my draft board said I had to come back and serve my military time. There was still the draft. So I came back. At Caltech I had made friends with a postdoctoral fellow, Alex Rich. He's still a very well-known professor at MIT. Alex got a commission in the Public Health Service because he had to serve his military time. And when I came back he offered me a position here at N.I.H. where I also joined the Public Health Service – satisfying my military requirement.

We were joined by another person who had been a postdoctoral fellow at Caltech, David Davies, who is actually still here at NIH, though he's officially retired. And we began to work just about that time.

UD: When did you start to investigate the interaction of regulatory proteins with DNA?

GF: Oh, much later. So now I'm here at NIH, serving my military time, and just at that moment Severo Ochoa and Marianne Grunberg-Manago synthesize the first polynucleotides. They are polyribonucleotides – polyadenylic acid, polyuridylic. They did that with the help of an enzyme called polynucleotide phosphorylase, which is not biologically significant for synthesizing; it's actually a degradative enzyme. It was un-templated, of course, unlike Arthur Kornberg's discovery of the DNA polymerase which required a DNA template and copied it, this enzyme polymerized whatever you put in. So Ochoa made polyadenylic and polyuridylic acids. Alex Rich knew Ochoa and as a result we got samples, and we began to study the interaction of these two polymers, which can make base pairs between A and U. They form a duplex – an RNA duplex. And then we discovered-

UD: What is the name of the duplex?

GF: PolyA dot polyU (polyA·polyU) – dot meaning forming a complex.

But what turned out to be really novel, and it was the first thing that hooked me on this field, was that we could bind a second strand of poly U to the duplex to form a triplex – a triple-stranded structure. That caused a lot of interest. I started by studying what stabilized these structures. I studied the phase transitions, because under some circumstances you only got the double-stranded version and in others you got the triple-stranded version. And I studied that not only when I was here, but also after three years, when I moved to the University of Pittsburgh. They had one of the few biophysics departments in the country at that time. I was an assistant professor there.

It became clear that because these are long chains that are negatively charged, ionic conditions in the solvent make a big difference to stability. If you want to make a molecule with multiple strands in which lots of negative charge is concentrated, you ought to have positive charge available nearby. Salt - positive sodium ions, for example, or better yet magnesium ions, will help to stabilize it. So I began to study how ionic conditions affected the stability not only of these synthetic structures, but also of real DNA. From there, the next thing was to use larger and larger positively charged ions and I did that. I studied polylysine and polyarginine, both peptides carrying positively charged amino acids and things like that as models of what might be going on in the nucleus. Because we already knew there were histones in the nucleus and they were positively charged, with lots of lysines and arginines.

2. Work on chromatin structure and its relationship to gene expression

And then one day - I had the cartilage taken out of this knee when I was about 42 years old in hopes that it would help. I remember that Shneior Lifson, the great theoretical chemist at the Weizmann Institute, came to visit me at home, and I told him that I was now working on the histones – I'd stopped working on polylysines and I was working on the histones. I said to myself, "If I'm going to say that this is a model for biological systems, maybe it would be better to work with the biological system." Shneior told me that I was making the right choice. And that's how that started. I purified histones and used them rather than synthetic polypeptides. That was the beginning of my interest in chromatin.

Actually, someone who had been studying histone/DNA interactions was Chargaff. I published my first paper in *Nature* on the subject, and I started it by saying that anyone who works in this field has to acknowledge that the starting work was done by [Erwin] Chargaff – it

was [Charles] Crampton, [Rakoma] Lipshitz, and Chargaff. After I published this paper on histone/DNA interactions I published another in *Nature*. And there I just referred to the first one. I got a letter from Chargaff saying, “I see that you did not cite my paper. I want you to know that I was doing science when you were still in junior high school." I wish I had kept that.

And so I sent Chargaff a letter saying I was sorry he felt this way, and I enclosed a reprint of my first paper, showing that I had given him very strong praise and citation. He never wrote back, which I thought was bad form. He should have apologized. But that was him.

However, from that time on, I became more and more interested in biology. And I began to be interested in the actual structure of chromatin and how the histones covered the DNA, and we developed nuclease probes to look at the chromatin structure. And then we hit on the chicken beta globin locus, as a perfect test system. We were physical chemists and you have to know that making chromatin in those days was more or less like Macbeth and the Witches Brew. People made chromatin from calf thymus; this was the standard way to make chromatin. The trouble was that this tissue is full of nucleases and proteases and you had, perhaps, three or four hours – it took a day to make it, and at the end of the day you started the experiment. You couldn’t let it sit overnight. And you never knew what you were getting.

At this point, I think it was Harold Weintraub (who is now dead, unfortunately, of a brain tumor and greatly missed) who for other reasons was beginning to study chromatin in chicken erythrocytes. Weintraub was interested in the chicken beta globin locus as a model locus for studying gene expression. Chicken erythrocytes keep their nuclei – birds keep their nuclei whereas we do not. And the nuclei are shutting down, really. But they still contain a lot of the active components and the main thing is that they’re almost free of nucleases and proteases. So it’s dead simple – you get blood from a chicken, you break open the cells, you centrifuge and wash a couple

of times, you have a beautiful preparation of white nuclei, and then you can do what you want. So you can study the chromatin of an active gene in a cell where you can hope for relative stability and resistance to degradation. Perfect for a physical chemist.

And that's what we had to do, and we first characterized one of the early enhancers that control the whole locus. The main thing, though, was that we were able to show that you could take chicken red blood cells –- and first treat them - change the solvent conditions and make pores in the cells. And then float anything in that you wanted, like DNA, nucleases, anything. That is at a time when transfection was a very difficult thing to do. People were just beginning to introduce DNA into eukaryotic cells. But this was just perfect – again for a physical chemist – it required no sophistication whatever. It worked every time, it was reproducible. Whereas I remember a colleague working in this area, who was discouraged because he was working with mouse erythroleukemia lines, looking at beta globin expression, but it was terribly irreproducible; he couldn't get the same result twice. That was a great advantage for us. Our interest in regulation led, ultimately, to our identification of the first of the GATA proteins, GATA1. We discovered it at the same time as Stewart Orkin at Harvard. And that turned out to be the founder of a big family of GATA proteins that are major regulatory proteins. GATA1 is very important in erythroid cell development. This was at a time when MyoD was being discovered. People were beginning to realize that there were cell-type-specific, developmentally-specific regulatory proteins.

UD: You started to focus on the relationship between chromatin structure and gene expression in eukaryote cells that you just mentioned. And you had a particular interest in the structure of both individual nucleosomes and polynucleosomes. I have a very stupid question. When I was a student, I learned about chromomeres. What is the relation between chromomeres and nucleosomes?

GF: Well, that's a much higher level of organization. Chromomeres are regions of condensed chromatin visible during meiosis and

mitosis. And they would certainly contain many nucleosome**s:** the nucleosome is the smallest scale organizational unit of chromatin, with about one for every 180-200 base pairs of DNA. We were doing so many different kinds of things at that time. The nucleosome was first discovered and characterized structurally and chemically by Kornberg and Thomas, and identified in the microscope by Olins and Olins, who actually had a cover on the front page of *Science* – showing this bead-on-a-string structure. Before that there were a lot of uncertainties about the histones, because there seemed to be a lot of them, but it turned out that many of them were just degradation products. There were really only five altogether. Four that formed this central complex called the nucleosome core, and one on the outside. The core contained an octamer of histones (two each of the four central kinds) - I don't think we need to go into the details of what they are. But around this octamer, two super-helical turns of DNA are wrapped. About 165 base-pairs of DNA, locked in place by the fifth histone. And that is the first order of compaction. Because part of the purpose of chromatin is to compact the DNA. So you get this bead-on-a-string structure, and that's what has been most studied. But it folds up further into far higher orders of compaction, and we studied the next order of compaction. That is still being actively studied, and it's still a matter of uncertainty. We don't do much of that anymore.

UD: Is there any relationship between the structural units and biological function?

GF: Yes. It's a good question. If you had asked me five years ago, the answer would have been much more certain. The thought was that these higher orders of structure were ways to compact the DNA that was not needed for function in a particular kind of cell. And that the structure was opened for transcription and that may still be true. But at the level of the nucleosomes themselves, it is clear they chromatin plays a major role. People began to ask whether the histones had to be removed to accommodate transcription, or of how chromatin structure had to be modified. At about that time Vincent Allfrey at the Rockefeller Institute, began to provide evidence that

histones associated with transcriptionally active chromatin were modified, particularly by acetylation of lysines.

I think most people paid too little attention to this. This was a mistake, and Allfrey deserves a lot of credit for persevering. But what really made the difference was that people working in tetrahymena and yeast began to isolate enzymes that modified histones or affected chromatin structure. So the problem always was, with Allfrey's results, chromatin containing active genes had acetylated histones, but was that just an accidental correlation? But with yeast genetics it was possible to show that mutating the enzymes responsible for histone modifications such as acetylation had a phenotype, a direct effect on yeast growth. Two or three groups really were able to show that. David Allis identified an enzyme in Tetrahymena that acetylated histones, and realized that it was similar to a protein in yeast, and it was known that when the gene for that protein was mutated in yeast there were effects on growth. That made the connection between the biology and the chemistry. Stuart Schreiber discovered an enzyme in mammalian cells that removed histone acetylation marks, and was related a yeast gene known to regulate transcription. And then there was the discovery of enzymes that move the nucleosomes around on DNA, which also had significant phenotypes detected through yeast genetics. That was a different class of proteins – chromatin remodeling enzymes.

Now the problem has been, for a long time, how to interpret the patterns of marks in terms of active state. When Allis first reported his work, he talked about the histone code, which would mean that there was a series of marks which could be reliably associated with either active genes on the one hand, or inactive genes on the other. To a certain extent one can identify promoters and enhancers as being associated with characteristic histone modifications. But gene activation is not so simple. And, in fact, you have to do it almost individual gene by gene. Each has its own way of activating, and each will have its own pattern of events leading to activation. In some cases, you completely mask the promoter with a nucleosome, and that tends to make it difficult for a regulatory protein to get in without

help from histone modifying and remodeling enzymes. In others, you let transcription initiation occur, the RNA polymerase gets on board, but then is paused and stuck, and then until you do some modification later, perhaps of a histone, it won't go.

So there are modifications that alter initiation, binding of RNA polymerase, and there are modifications that alter elongation. And there are modifications that affect splicing - our RNAs are spliced if they have introns. And many are alternatively spliced. There are many genes with multiple introns, and the actual final RNA product will not use all of them, but a subset. And under different circumstances, a different subset. So you get different proteins out of the same coding region. And that also is controlled in part by these epigenetic marks. But this, of course, doesn't address the question that I addressed in my review, which is whether this is truly epigenetic or really counts as just a part of the biochemistry—although a very complicated part and an essential part. So the point of view I was trying to express in the review is that, first of all, most of these are not epigenetic. The rather loose use of this term has roused such animosity among the people who think that this is just a fashionable term to use.

UD: So when you say they are not epigenetic, that means…

GF: It means that they are not marks that are transmitted through cell division or the germ line. The point is, that it doesn't make any difference from the point of view of the science because whatever you call these things, they are the mechanism for the regulation of gene expression, and that's what you have to study. So you can call it anything you like. It's too bad it got called epigenetics. Because this implies something about inheritance, and the role of the environment, and we are still a long way from understanding that.

UD: Yes, you expressed this in your review and suggested to go back to the 1942 definition of Waddington.

GF: Yes, why not? Now we really know what we're talking about.

UD: But the question remains—how are these mechanisms controlled?

GF: We don't know. They are controlled in as many different ways as nature can accidentally devise. One of the things we're finding out, is that in fact nature has tried everything. Almost anything you can imagine in the way of a mechanism is beginning to turn up. Because evolution is, as I always say, not constrained to – not being told to write a textbook in which 50 pages will be devoted to epigenetics, and we better get it all in there. It keeps writing new pages. And there are no rules – except the rules of chemistry and physics.

So, as I said in the review, the first thing that has to happen in the control of gene expression is that you have to have a transcription factor that recognizes a DNA sequence. In the promoter, usually. Sometimes an enhancer, but it has to be an interaction between a protein that actually recognizes a particular, and usually unique, DNA sequence. That is the initial step. That carries the information. But once that happens, anything can happen. The next thing may be that you recruit a nucleosome remodeling enzyme for the nucleosome that is next door, which pushes that nucleosome out, and now the next factor can arrive, and then that factor may further recruit histone-modifying enzymes. That loosens the chromatin structure, perhaps. And finally the RNA polymerase binds and will recruit further histone modifying enzymes. So as it moves along the gene transcribing it will modify the nucleosomes – maybe loosen them up so that some of the histones can jump off and then back on again – not necessarily the whole nucleosome, just a subset of the histones. But each gene will be different and the order of events could be different under different circumstances or different kinds of cells.

UD: But still, what is behind these events – let's say first there is the regulation of the production of the first transcription factor, which is encoded by a certain sequence…

GF: So how did all that happen?

UD: Yes.

GF: Well, we have to go back to the pluripotent stem cells. The answer beyond stem cells – there's nothing to say at the moment. They are the product of evolution, of course. But we are just at the beginning of tracing the evolution of the regulatory networks that constitute the program of a pluripotent cell, and the differentiated cells it gives rise to. The stem cells come equipped with proteins like NANOG. This is one of the central proteins that maintain embryonic stem cells in their pluripotent state. It and other factors maintain the pluripotent state, in which some genes are of course active and others silent. Some genes appear to carry histone marks for both active and inactive genes – presumably so that they can go in either direction depending on which kind of specialized cell they become associated with during development. Departure from the pluripotent state is associated with disappearance of NANOG. That allows other genes to come on, and still other genes to switch off. Depending on the stimulus which comes into the cell, that cell will go along a pathway towards neuronal development for example, or development into any other kind of specialized cell. That will involve, then, a change in the array of factors that's expressed.

Even though sea urchin development is quite different from vertebrates, the underlying scheme is really like what Eric shows with his detailed analysis of programmed development. In both cases this factor turns on and binds to this promoter, which turns on this gene, which represses another promoter which turns off that gene, and suddenly you're no longer a pluripotent cell, but you are turning into a fibroblast, for example. That is what Yamanaka got his Nobel Prize for last year. He took a somatic cell and expressed, a small group of factors including NANOG. There are subsets that will work. Probably because it is cyclic. Once you establish this circle – this acts on this, act on this, acts on this, it comes back on itself. So once you establish part of the circle, you may be able to get it going and then it is self-sustaining. So we don't have a first cause.

UD: The first cause is the DNA?

GF: I was thinking about establishment of a pluripotent cell given that the mechanisms already exist. But in terms of evolution it's probably RNA to begin with. There are many functional RNAs. It has turned out that not only proteins are involved, but non-coding RNAs which are protein-like. They can be thought of as being just like proteins. They have enzymatic properties, and they interact with proteins. And it is sort of amusing because in the lab, in the last four years or so, we have been interested in a protein that is complexed with a long RNA, which has regulatory properties. When we started to work, we thought of it as if RNA is helping the protein fold so that it can function as an enzyme. But it may very well be that the protein is helping the RNA to fold so it can function as an enzyme. So we changed our point of view.

One of the things that we're interested in now is connected to our previous study of the chicken beta globin locus. Very early on we had mapped the histone modifications over the locus and made one of the early identifications in a vertebrate of histone modification versus activity. We noticed that this entire beta globin locus, which contains all four beta globin genes that are developmentally expressed either in the embryo or adult, was embedded in a whole bunch of silent chromatin. The question was, why didn't the silent chromatin spread and swallow up the active, and why didn't strong enhancers located nearby turn on the globin genes inappropriately? So we began to look for boundary elements, something that would keep the wolves away. We found that there was a region just at the edge of the beta globin locus that contained some proteins that blocked the advance of silent so called heterochromatin. And we also found a protein called CTCF which, when bound to DNA, keeps enhancers that are outside, or any other activating signal that is outside, from getting in. CTCF was a known protein but had not been identified as having that function. This kind of protein is called an insulator.

It became a major object of study over the last 10 years for a large number of scientists. And we are included. It turns out that if you have a CTCF bound to DNA here and another bound at a distant site, they can find each other over long distances. They form a loop. And indeed, if your gene is in inside the loop and your enhancer is outside, it will keep the enhancer from reaching the gene, which will tend to be silenced. But there are other situations in which the enhancer and promoter are both inside the same loop and then it tends to bring them together, and the gene may be activated. And so it turns out that CTCF is the foundation of a lot of large-scale nuclear organization that is designed to help regulate long-distance interactions in the nucleus.

So now, lots of people are studying long-range interactions in the nucleus and how they affect gene expression. This is made possible largely because of a discovery some years ago by Job Dekker of a method for mapping physical contacts within the nucleus. This nuclear architecture is essentially established right after cell division. Very recent studies by Dekker and his collaborators show that the structure is disrupted and a new on established in mitotic chromosomes. But once you get through cell division, then the whole genome is in loop domains – on average perhaps a megabase in size. And in addition genes with related functions, even on different chromosomes, will often tend to bunch together.

UD: What does related functions here mean?

GF: For example, the beta globin genes and many other genes in erythroid cells are in part regulated by the transcription factor Klf1, and these tend to cluster together, as Peter Fraser has shown.

UD: It sounds like a miracle; how would they recognize each other?

GF: Well, we don't know. The net effect is that genes that require a specific factor will find it concentrated at the appropriate cluster. But I think it is still not clear whether the concentration is a cause or an

effect. That's what people are interested in now. That's the level at which all of this is now being explored.

UD: That sounds really, really fascinating. Can I ask you a question about research that was not so successful? Were there any major dead ends in the research on chromatin structure that you remember?

GF: Actually, we didn't have many. Except we did not see the nuclear sub-structure, I have to say. We weren't thinking that way. I was thinking in terms of the biology and not the structure at that point. It was a mistake. You have to think about structure before biology.

People tried to study the properties of the individual histones. They even solved crystal structures. And these turned out to be of no interest whatever. Because those histones never—in vivo—ever exist, except as part of a complex with other histones or with chaperones.

UD: So they isolated them, crystallized them, and it didn't lead anywhere?

GF: No, they form interesting structures that are irrelevant.

And there were a lot of experiments trying to transcribe chromatin in-vitro, isolate chromatin and then show that the transcript you got was restricted, as you might expect. We did some like that, and I think ours were OK. But a lot of it was actually looking at endogenous RNA which was there because you couldn't purify it away. RNA was actually part of the original chromatin rather than something that was created when you tried to transcribe. Somebody who worked with me at the beginning of that was Chaim Cedar. He's at Hebrew University, Hadassah. He has won the Israel Prize, the Wolf Prize and the Canada Gairdner Foundation Award. You might know of his son, Joseph, who has made these beautiful movies. 'Footnote' was a recent one. Chaim and Richard Axel were post-docs in the lab at the

same time. Axel won the Nobel Prize for his work on the odorant genes, the sense of smell.

UD: I have another question which refers to what many people say. Is there any evidence for soft inheritance through epigenetics?

GF: You mean, I ate a bad diet. Will my offspring…? Well, I thought there was some – there are strong correlations between parental diet and ultimate susceptibility to diabetes, I think. There are people who study that kind of thing.

UD: In mice.

GF: Well, and in humans also. There's someone at Cambridge who's actually doing that.

UD: But it doesn't sound as if it had a selective advantage.

GF: No. But most mutations, if you like, epigenetic mutations, shouldn't have a selective advantage any more than any other mutation does.

UD: Right, but Lamarckian inheritance is supposed to have a selective advantage, and I'm asking whether there is any evidence there.

GF: No, I don't think so.

GF: Well, there is evidence in mice, for example, that if you mess with their DNA methylation that has an effect on offspring that can go down through generations. It's DNA methylation. And the same is true in plants. But it is metastable. So it will last for three or four generations, or five generations, and then it gradually dissipates because the methylation patterns are not so faithfully copied.

UD: And what is the advantage for it?

GF: Adaptability in some cases. Rapid adaptability, could be. And I don't think I mentioned it in my written article, but Susan Lindquist has extensive data in yeast—concerning how epigenetic changes can

actually help to stabilize, under selective pressure, genetic mutations. They lower the threshold for the survival of mutations to the point where the mutations eventually take over and replace the epigenetic mutations, if you want to call them that.

UD: It seems to me that here, too, the effect is random; the epigenetic changes are not adaptive not is it specific mutations which are stabilized, but all of them. Is this correct?

GF: Epigenetic changes may for example reflect environmental changes, but I don't know that they are any more adaptive than classical mutations. To the extent that they are transmitted through the germ line they could be selected, but there is only a little evidence for transmission through many generations

UD: I would like to thank you very much for sharing with me your scientific experiences and views.

GF: I just remember something that profoundly influenced me when I was young. I was born in the United States and my parents were born in the United States, but all my classmates were from Germany and Austria. I grew up in a part of Manhattan, West End Avenue in the 90s, which was apparently very attractive for people who grew up in Vienna. I discovered it when I visited Vienna, because it looked a lot like some parts of Vienna. A very large fraction of my classmates had fled. And they came with an education of a kind I had no idea about. We were years from reading our first Shakespeare, but I think they had read a large fraction of the plays already in German. They knew everything. And realizing what there was in the world to learn probably gave me a jump start of years. I remember particularly one thing that annoyed me. They came in on a Monday all excited. They had been to see this wonderful play called Oedipus Rex. I'd never heard of it. And they had seen it with this wonderful new English actor, Lawrence Olivier, of whom I'd never heard. And as I got a little older I realized what I'd missed – there was a lot to learn. But I think those influences were very important.

UD: Thank you again very much.