My name is Michael Mark Gottesman and my position is deputy director for intramural research at the National Institutes of Health.

I was born on October 7, 1946 in Jersey City, New Jersey. And when I was around two years old, my family moved to Flushing, Queens, and I had most of my formative years growing up in Flushing. I cannot remember a time when I wasn't interested in science. Probably the first interaction with issues related to public health was as one of many probably millions of children in the United States who got the Salk vaccine as a -- as a test. I remember lining up, they explained to us that this was a trial, and we all got shots, which was not that much fun for a six-year-old or a seven-year-old. And that was a huge sea change. I remember learning about the fact that before then people got polio, kids got polio. They wandered off to camp, they came back paralyzed. And after that period, we didn't need to worry about polio. So I had the sense that there was a lot that biomedical research could do to alleviate human disease. The next big event scientifically in my life was the launch of Sputnik in 1957, and it was a wake-up call to the United States. We were so-called "falling behind" in the space race, and I was an eleven-year-old boy who was interested in space science. So I spent my childhood after that making rockets, probably not as safely as it should have been, but no unfortunate accidents befell me. And I remained really interested in science. Initially chemistry and then more so biology. And I remember when I -- I skipped a couple grades, so one interesting fact about the first three Genome directors was that we were all out of high school by age 15. I don't know if you realized that. Francis and Jim and I all graduated from high school at a very early age.

So I went to college, Harvard College, at age 15. I was the youngest person in my class, but also still extremely interested in science. I majored in biochemistry as an undergraduate, and probably the first course I took was Biology 2, and Jim Watson was one of the professors in that course, so I was exposed to him at a rather early age. And Jim was a character even then. Obviously really smart and entertaining, and very liberal in his comments about other people.

I don't think he was very organized about his lectures. They were mostly reminiscences. But he covered the material by talking about the people and what they had done, what contributions they had made.

Jim has a funny interpersonal style, as you know.

But he was forthright in talking to the audience, not just mumbling at the blackboard.

So let me tell you, as an undergraduate I met my wife, who played an extremely important role in my life. She was a year behind me at Radcliffe but was actually a bit older because of my young age coming into college. And she was interested in either molecular biology, which was just getting to become a discipline, or in social sciences. So she had a sort of broad humanities as well as science perspective, and we started going out together when I was a sophomore and she was a freshman. And we've remained together ever since, so you might figure out we were married when I was a senior in college, so we're about to have our fiftieth wedding anniversary.

Oh, that's lovely.

It is lovely. It was a -- it's been a wonderful relationship. And we share a lot in terms of our interests. She I would say is much more oriented towards very basic science, particularly microbiology, microbial genetics. And she helped me early on to appreciate those disciplines and we've had lots of conversations about science over the years, as you might imagine. But one interesting aspect of her undergraduate career is that she spent time in Jim Watson's lab. You know, Jim Watson had this reputation for hiring Cliffies, Radcliffe students, and Susan was one of many who worked in his lab. She didn't work directly for him, she worked for someone named Gary Gussin, who was a graduate student in Jim's lab. So in addition to having him as a professor, which was a somewhat distant relationship, I knew Jim a little more not so much socially, but I knew him -- I would go to see Susan in the lab, and Jim would be there. So he knew that we were a couple. And later on, when we went to Cold Spring Harbor meetings, which were at least annually of various types, I got to know Jim reasonably well. So I knew Jim at the time when he became director of the Genome Project, and he knew who I was.

The course that I took called Bio 2 was taught by four professors, one of whom was Jim, one of whom was E.O. Wilson.

So I should say that as a biochemical sciences major, I had an opportunity to have a tutor and write a thesis, and a I had a publication, my first publication, and I worked with a scientist named Bill Beck who was a hematologist at Mass. General Hospital. And the most exciting part about that was that the cold room that I had to put my material in said "Lipman" on it, it was Lipman's shelf. So I knew that I was among great scientists there at the Mass. General. I also worked -- I spent a little time working with Guido Guidotti, who's quite a well-known biophysicist at Harvard. And I went off to medical school, Harvard Medical School. There was a period of time, because Susan was a year behind me, she was a junior -- we were married in my senior year, it was clear that we wanted to stay together. I know it's common now for couples to separate, but we didn't want to do that. And so we wanted to find a graduate program for me and with the potential of that being true for her as well. And I applied to not that many different medical schools and got in to a number, but decided that I really wanted to go to Harvard because that would allow Susan to stay in Boston. At that point it was clear she was interested in bacteriology, and she ended up working with Jon Beckwith. And we lived initially in Cambridge when she was an undergraduate finishing her last year. And actually, I don't know if you know a Josie Briggs. Josie Briggs is the director of what's now called NIHCR. And Josie and I were classmates at Harvard Medical School, and she lived in Cambridge as well, and we used to carpool together, and we shared a cadaver in anatomy, actually, so.

And in fact, there are a number of people here who had Harvard upbringings who I got to know reasonably well over the years because we have similar kinds of backgrounds.

And one of them is Bernadine Healy. So Bernadine was not in my class to begin with. She took a year off to work with Bernie Davis, who was a bacteriologist with Harvard Medical School. But we graduated together. Now, in medical school, there's a fellowship of people, at least at Harvard Medical School, because we were all arranged alphabetically, I got to know the Fs and the Gs and the Hs extremely well, because we were all in the same labs together. But Bernadine was not there for the first two years, so I really didn't get to know her that well. She graduated with me and she was kind of somebody in the class, but not a close personal friend or anyone I knew very well.

So potentially, yeah, because I never quite understood why I was chosen to be the acting director of what was then NCHGR, National Center for Human Genome Research. I think Bernadine knew me sort of vaguely and appreciated that we had been in a class together, but we weren't close friends.

So I got my M.D. in 1970. So I had a perfectly linear education. Four years in college, four years in medical school. In 1970, every single physician who graduated was drafted to go to Vietnam or equivalent service. So we all appreciated that in order to avoid being sent to Vietnam, we needed to find another alternative public service. And fortunately, there was the Public Health Service as an option, and you could -- you could go to the CDC, you could go to the NIH, you could go to the Indian Health Service. And I -- since I was interested in research, I had already begun to do research as an undergraduate, and in medical school I worked with Bert Vallee, who was a biochemist at Harvard Medical School, and I had published already two or three articles by that point, and decided that I would go -- come to NIH. And my advisor in a lot of these things was a fellow named Robert Simpson. Bob Simpson was -- then, he was an M.D. who was getting his graduate degree in Vallee's laboratory, and he was my direct supervisor. Wonderful mentor, very interested, knew everybody at NIH, had spent some time there and was interested in helping me find a place to work. And so when I came it NIH-- this was, I should say that this was after I did my internship. I did a medical internship at Peter Brent Brigham.

The first time I came to NIH was actually in '71. So I graduated in '70, I did a year of medical internship, and then I came to NIH for three years, and then I went back to Harvard, where I was an Assistant Professor in the Department of Anatomy. And I finished my senior residency, I spent a year in the faculty at Harvard, and then I left. Much to their chagrin, because they told me that nobody at Harvard leaves after one year. They usually make you suffer for seven or eight before they allow you to leave [laughs].

So I left after a year, and a lot of that was Susan's doing, because she was at that point postdoctoral fellow work with David Botstein, and we had an opportunity for two quite good jobs at NIH in 1976. So both of us became Senior Investigators very quickly after we arrived. Currently, the process which I've instituted as DDIR is much more selective [laughs]. So it wouldn't have been possible under the current regime to do what we did [laughs] that many years ago.

So we were -- we were both recruited by Ira Pastan who I think primarily was interested in Susan because she had done a postdoctoral fellowship in the laboratory that he was chief of with another Gottesman, Max Gottesman. So we were recruited back. He definitely wanted Susan, and he thought I didn't look too bad on paper, he would recruit me as well. And we were both set up with all of the trappings of tenure-track Investigators with research resources and told to do the best science we could possibly do.

Right. So initially when I was a postdoc at NIH in 1971, I worked with Marty Gellert. I was what was called a research associate, and that was in what was then NIAMD. It's now NIDDK. And Marty was a fabulous mentor. He's a terrific scientist. He's still a very active scientist. And I probably learned really the most about science in that laboratory. IT was great. And then when we got recruited back by Ira, we were already ready to be somewhat independent. And you know,

given that I didn't -- don't have a Ph.D., I have an M.D. -- having had a number of these research experiences under really good circumstances I think made a huge difference for me.

Well -- so I think I was partly influenced by my wife, who is as I said at core a very basic laboratory scientist. I think I appreciated also we started to have children, and I wanted some time for family. We talk about in medicine about a "triple threat," somebody who practices medicine, who teaches, and who also is a superb clinician. And I knew that it wasn't possible to do those three things and also have a family. And so the clinical work I pretty much gave up. But I was really interested in laboratory work, which had the advantage of flexibility of hours as well as sort of intellectual stimulation. So I focused on that, and I think you know I've still retained a firm footing in the laboratory.

So I've asked myself that question on many occasions. So it you know for a couple years before then, Jim had been actively lobbying the Congress and his scientific colleagues to put aside some money to basically sequence the human genome. It was a vision way ahead of most scientists. And I would say I was in the category of most scientists who thought who thought that experimental science could proceed perfectly well without an entire human genome sequence. Even though I was interested in both human and mammalian genetics, somatic cell genetics. I would say I was not at that point a major proponent of the concept that you could take money that could be used for other research purposes and create an infrastructure that would last you know forever. So I was not a strong proponent of the Human Genome Project. And most of my colleagues were not. Jim's position was I would say pretty unpopular with most scientists, and they viewed it of course as unfortunately possibly taking money that could be used for other purposes and putting it into this big structure. Obviously time and history have proven that this was a truly visionary investment. And Jim was the moving force, there's no question about it. He spent a lot of time meeting with Congress, he had a Nobel Prize which made him credible, and he marshalled very strong arguments about the utility of doing this. And so the project was developed. Obviously you know the history in terms of the DOE and other people who contributed to the concept, but Jim really was the major force. And he -- as I remember, he spent a lot of time still at Cold Spring Harbor and would appear occasionally to sort of right the ship and get it heading in the right direction every week or every two weeks, I don't remember exactly how often he was there. He had the foresight to hire Elke Jordan as his deputy. Elke is fantastic. And she was -- she was everything he was not. She was organized [laughs]. She was capable of marshalling the resources she needed. And she also though was I think a strong supporter of his vision. She agreed with what he wanted to do. She helped to hire some of the people like Mark, Jane, and so on, Jane Peterson, who were important components. And he also had a vision which I think occupied not a small amount of my time when I was acting there for almost the year, of having an ELSI program, Ethical Legal and Social Implications of the Genome. He appreciated that this was going to be a really important issue in the future. So both in the concept of having a Human Genome Project and then how it would play out in the larger social arena, he was very prescient I think. Amazingly so.

So I am not privy to all of the discussions behind the scene obviously, but it was clear that Jim and Bernadine were not getting along at all. There were -- there's probably public record of things that Jim said about Bernadine, and she took this not only as personal insults, in many cases they actually were, but as a challenge to her authority. And she couldn't -- really could not tolerate that, and

made a decision that Jim should no longer be director of the Genome Project. And before that was announced, she wanted to have in place an acting director. At that point, she had an acting deputy director, Carl Kupfer, who was then also the director of the Eye Institute, long-term director. And Carl called me up and he said, "Dr. Gottesman, can you come to Building 1, I need to talk to you about something." And I thought, "Oh, my God, this is -- the principal is calling me to his office" [laughs]. At that point, I was the director -- so I was the chief of the laboratory of cell biology. I had already become chief of the laboratory of cell biology in the Cancer Institute. So I had some credentials, and he said, "Well, you're a geneticist, and we need somebody to run the Genome Project." And I said, "I'm not [cell phone pings] really a geneticist, I'm a somatic cell geneticist" [laughs]. "If you want a real geneticist, you should look elsewhere." And he said, "Well, Dr. Healy thinks that you would be a good director of this." Now, I was a little bit known in Building 1, which Building 1 being the central NIH, because I had been involved in an effort to develop a summer student program, and I had worked with some of the people in Building 1 to get the resources together to support students in the summer. So I think it was clear to people at that point that I had a sort of broad trans-NIH perspective on training and scientific issues. So that may have played a little bit into it. I suspect also there were elements in my personality that were attractive in terms of the program because there was -- there was a lot of turmoil. Jim leaving was going to be a big deal. He had a very dedicated staff. He had handpicked the people on the staff. And they really worried about morale. And so they wanted somebody who they thought could be a caretaker who would apply a personal touch to make sure that things stayed on track.

And I also -- I think I took the job with the understanding that it would be a temporary position, so there was no ambition --

So I saw myself basically as a caretaker, but "care" in the best sense of the word, to take good care of the program and begin the process of recruiting a new director, which began almost immediately. So I had two priorities, two major priorities. One was to make sure that the program could continue to work well. The major plus in terms of that was Elke, who was already running the program most of the time anyway. But I dealt with morale issues. I met with the staff on a number of occasions, I assured them that their jobs were secure, that they can continue to do what they were doing, that I would provide the guidance that I could, and you know a director of an institute or a center is involved in personnel issues and all kinds of things that are day-to-day issues, and I tried to reassure people that I was comfortable in helping them through this transition. That it was a transition. And that we would find a good permanent director.

Right. So because I was intending not to be there forever or even for a long period of time, I didn't see the need to visit each of the centers. But I visited a couple of them. I went out to California, and I actually spent a fair amount of time at Washington University, which is where I got to know Eric and Bob Waterston. And that, at that point, was probably the most active sequencing center that we had. And recall in those days, the sequencing was pretty primitive, right [laughs]? Sanger sequencing, or -- so the couple of visits that I made were really opportunities to educate myself about what the technology was, and to help formulate -- I mean I didn't want the program to lack leadership, but I wanted to move as quickly as possible to long-term leadership. So we had -- I had some priorities. I mean, one of the things we were very concerned about was the need to develop the technology, early on in the program, to develop the technology that would actually enable sequencing 3 billion you know nucleotides. And we did not have the technology to do that.

Certainly for not for 3 billion dollars [laughs]. So a lot of the early grants were in fact technology-based grants, and I remember enjoying reading the very clever and unique ideas that people had about how you could speed up sequencing. And then the issue became one of demonstrating that the sequence could be useful. And what was closest to being clear was that the yeast sequence was not yet completed. The Europeans had started it, and Bob was very interested in continuing it at Washington University, and so we -- I should have pushed to make sure that the funding was adequate to make sure that yeast got sequenced. And of course, if I can take some credit for the yeast sequence I'd be very proud because I think that really was the first demonstration of the utility of having sequence information of a whole organism.

And we also started the concept of having this be an international collaboration, that it wasn't, oh, we're going to be the Genome Program in the United States, but there are lots of other partners with whom we could work. I think that was extremely helpful.

Well, I think probably the most significant thing was the demonstration that the sequencing of a complete organism like yeast or the work on C. elegans could lead to a quantum leap into understanding the genetics of those systems. And I think there were still a lot of people -- remember I said I came in not being entirely convinced. I left after the year being convinced that this was a really valuable contribution to science. And I hope that during that year, there was a switch in understanding of the scientific community that this would be a worthy investment of Federal funds. So I think if -- I can't take full credit for that, but there was a certain momentum to the program, and during that year I think there was a switchover in the positions of many scientists about the value of the program.

Right. Yeah, I -- and I don't -- what I remember about the relationship with Craig Venter was that there were instances after Francis became director -- and I think it was after I was even acting [unintelligible] director where, because of my sort of unique position as having been an acting director at the time of Craig transitioning to TIGR, where I tried to be a moderator or a neutral party, and there were a couple meetings in which I was trying to translate, simultaneously translate what Francis was trying to accomplish and what Craig was trying to accomplish to get the two groups together. I don't know that I was that successful in that, I don't think there's been a longstanding amity that resulted from those relationships [laughs].

I think it was a mix. I think their personalities were very different. Francis was a much more open, public kind of figure. And Craig had chosen a sort of more private, industrial route to get to his end result. There were arguments about providing information publicly and how that should be done. There were differences in the public pronouncements made by TIGR and by the Genome Project. And there was initially a huge difference in the approach that was [unintelligible]. So the Human Genome Project was sort of this organized "we'll sequence, we'll work on one chromosome at a time, and we'll put things together." And Craig had conceived this idea of shotgun sequencing and assembly, and there was skepticism. Craig was actually right that you could do it this way, but there was skepticism and that led to I think some sort of conflict.

Well, I -- so remember I was an intramural scientist. So what was significantly different was I was significantly different was I was dealing with extramural grant processes. And so I had to learn a lot about -- just the alphabet system of grants. And Elke was very good in taking care of those

things and teaching me about them. So for me, that was the biggest switch. The actual dealing with people, getting them motivated, keeping morale up, and so on, I think came sort of naturally. And I had done a little bit of that, because I did run a sizable laboratory in NCI. So that part of it I don't think gave me any pause. It was learning the mechanics of running an institute.

Well, I think that decision was Bernadine Healy's. I mean I -- but I had -- I had taken the job under the -- with the understanding that my position would be relatively temporary.

So there were -- as for many people at NIH, there were occasions when Bernadine Healy and I did not see eye to eye. And there's an interesting episode that, I don't know if it's recorded, in which a grant had been given that Jim had signed off on and the Council had reviewed and so on so forth to -- I can't remember the fellow's name, but he was a lawyer who was interested in criminology.

It was Wasserman.

Eric -- Eric -- Wasserman -- David Wasserman.

David Wasserman.

Yeah. That's right. And -- exactly. So this was a grant as part of the ELSI project. And I had to say that ELSI occupied a fair amount of my time, because there were always issues coming up about legal and social aspects of the Genome Project. And what Bernadine wanted to do was, she wanted me to withdraw the grant because she didn't think that using genetic information to determine whether somebody was guilty or not was a subject that should be discussed by the NIH. So the -- he wanted to have a meeting, and it was a grant to support a meeting in which there would be people talking about would for example the defense that "my genes made me do it" ever be a legally admissible argument that would lead to people being acquitted.

Exactly. And she wanted me to withdraw support for the meeting, and I talked to my colleagues and it was clear that a meeting which had been funded completely had never, ever, ever at NIH been withdrawn for what might be seen as political reasons. And so I said I just couldn't do it. So she did it. As director at NIH, she could do that.

I mean, it was very traumatic for everybody, and we felt that it really undermined the intellectual freedom that we were supposed to be supporting.

She was enormously talented as a speaker. She was compelling. I think she was very smart and hardworking. And she was outspoken, and I think a wonderful anecdote that I think puts everything in perspective is, I was there when she -- one of the first times she met with the institute directors at the NIH. And she walked into the room, and she looked around, and she said, "My goal at the NIH is to increase the average IQ and decrease the average age of the people who sit around this table." Now, you can imagine the effect on her colleagues [laughs], her saying that to a group of institute directors.

So in 1971 I had finished a year of medical internship and unlike many of the people who were physician scientists I chose to leave in the middle of my internship at the end of my first year of

internship and come to the NIH. Most of the so-called yellow berets, the people that came to NIH during the doctor's draft completed a residency before they came to NIH. I chose to complete an internship and leave because I was very anxious to get back to laboratory science, which I had started, in college and medical school. In addition it was a time when my wife had just finished her PhD and she was looking around for postdoctoral experiences and NIH beckoned. So, what happened at that point was that I was chosen to be part of this program at NIH called the Research Associate Program. And there were clinical associates and research associates so some physicians were oriented much more towards clinical studies and some towards laboratory studies and I chose to be in the basic science arena. And we had to choose an institute and looking around and based on the advice that I got from people up in Harvard who had been at NIH -- particularly Bob Simpson who I mentioned last time -- I thought the institute which was then called NIAMS, now called NIDDK would be the institute that had the most basic science. There already were active Nobel laureates working there and there was a lot of evidence that that was an institute -- which we've called in the past the General Medical Institute but really was a very basic science institute. And I was invited down for interviews and I met with a number of people. I talked to Marty Rodbell who of course later went on to win the Nobel Prize for his work on g-protein coupled receptors. I talked to Marty Geller. I talked to Maxine Singer. And I remember that they were all enthusiastic about recruiting me into the lab because the positions were centrally funded and I was a freebie. Even though my research experience was relatively minimal. I had had maybe the equivalent of a couple of years of research. It wasn't even at the level probably of a PhD at that point in terms of total experience. And I remember talking to Marty who is a wonderful scientist, a very astute scientist, a scientist's scientist -- and he was the only one of the three or four people who interviewed me who was not that enthusiastic about inviting me into the laboratory. He had a very small lab. He never had more than one or two post docs but there was something about him that really attracted me. I liked the way he thought about science. I thought I would gain very rigorous scientific training in his laboratory and so I joined that laboratory. Now, Marty was known at that point for having been one of the major discoverers of DNA ligase, a really important enzyme for putting DNA together. And later went on to do the same for DNA gyrase. So, he was really an essential part of the biochemistry underlying what later turned out to be the genome project. And Marty and I sat down when I arrived and we talked about what would be interesting for me to do. And I had mentioned already that I had an orientation towards bacterial genetics because my wife was a bacterial geneticist and I heard about bacterial genetics all the time -- the awesome power. Then it was of studying E. coli. Later it became yeast and I wanted to be -- to harness that awesome power. So we talked about a genetics project and the one that seemed most obvious at that point was to obtain mutants in DNA ligase and E. coli and see what the effect would be on the two processes that we thought would be affected in ligase mutants. One would be DNA repair, obviously, you have to cut out a bad piece of DNA, replace it, and ligate it back in. And DNA replication because at that point it was known that there was a leading and a lagging strand in replication and one of those strands required synthesis of DNA in segments -- Okazaki -- so-called Okazaki fragments that would be linked together. So we didn't know that the limiting feature would be. We suspected that an important element of this would be the fact that the mutants themselves would be lethal and we needed conditional mutants. So, we started to isolate mutants. We used a phage system in which the T4 ligase was missing and we needed ligase to function. And we isolated number of different mutants, one of which turned out to be a conditional lethal mutant in DNA ligase. And I began to study it and I published a few papers on the fact that that Okazaki fragment pair was limited, that they were U.V. sensitive as expected because DNA repair

was limited. And I got a sort of basic background in DNA enzymology basically in Marty's lab. After a year or so of working in the lab Marty decided he needed to take a sabbatical for a variety of reasons, some person, and he went to Europe for a year. And I was at that point one of the senior people in the laboratory so I was pretty much free to do whatever I wanted to do and I began to collaborate with some of my colleagues in the laboratory of molecular biology. I should point out that we were all in the laboratory of molecular biology in NIAMS, which is sort of a world-renowned laboratory. Currently has, you know, four or five members of the national academy and has always been preeminent in terms of the biochemistry and the studies going on in the lab. So there were a lot of other people around who could mentor me and help me. One of whom was Gary Felsenfeld who is just down the hall. Tommy Zao [spelled phonetically] who is a very well-known Japanese biochemist was at NIH at that point and it was -- it was exciting. So I had -- I decided -- and this was partly at Marty Geller's suggest that I should learn to do electron microscopy, that that would be a good way to study elements of DNA function and structure. And so I learned how to do E.M. and particularly did header duplex mapping. And I began a study with Lee Rosner who was another bacterial geneticist in the laboratory who was interested in antibiotic resistance in bacteria. And at that point it was known that genetically these antibiotic resistance elements could move from one genome to another genome within bacteria. And we became interested in studying chloramphenicol -- translocon. And using these EM header duplex mapping systems we were able to show that the -- you could move the chloramphenicol resistance element from a bacterial genome to a phage genome and so on and so forth. And we could actually map the size of the DNA fragment, which was moving. And that was my first -- sort of first taste -- and actually one of the very earliest studies on the mechanism of movement of antibiotic resistance genes. And I think in the back of my mind it was the seed that led to my later interest in drug resistance and cancer.

The other thing that happened in that laboratory is we used to give lots of seminars and journal clubs and so on and so forth. And Marty suggested probably because I am an M.D. and probably the only M.D. that he's ever had in his laboratory I think. There have not been a lot of others. Towards the end of my time there Mark Guyer arrived in the laboratory. I don't know if you realize this -- Mark was post doc in Marty Geller's lab. Kiyoshi Mizuuchi was in that lab. Kiyoshi is a world-renowned single molecule biochemist who is also a member of the national academy. And John Little was in the lab, a well-known phage biologist. So there were occasionally one of two other sort of senior people in that laboratory. It was a great place to think about science and do science and everybody was in their own domain thinking about what interested them most of all. The direct antecedent of that laboratory was Gordy Tompkins's laboratory and in fact my cold room shelf said Tompkins on it so, I went from Lipmann at Mass General Hospital to Tompkins at NIH. So, very indirectly I had people's space that they used to occupy in those two places. So among other things, Marty said to me while, you're a physician, and there's this new field of somatic cell genetics, why don't you learn something about somatic cell genetics and do a general club? So I read a couple of papers by Ted Puck, Jennifer's father. Who was a geneticist, Jennifer Puck's father -- Jennifer who obviously played an important role in genomics at the NIH later on. And the papers basically defined the use of Chinese hamster ovary cells as a very good cell line for doing genetics. And the main reason was they were sort of functionally hemi zygotes. They weren't haploid cells. They were somatic cells. They were diploid, but for many of the genes in the genome one of the two copies had been inactivated by a variety of mechanism I think still not fully understood. So it was relatively straightforward to get recessive mutations because there was

not a second gene to cause problems. And later on when I came back to NIH I decided to use that system to develop mutations in genes that affected response to anti-cancer drugs. So, the seeds of both my interest in drug resistance and my interest in studying cultured cells I think happened in Marty's lab and I attribute to Marty his suggestions, his advice, his mentorship that led to my getting into both of those projects.

I don't think so. So, one of my current jobs is to oversee the research activities at the NIH and I think curiosity driven science, a very basic understanding of biological systems, and it doesn't really matter what the system is so long as it's tractable, you can do good experiments, good experiments, good, controlled scientific experiments that give you information that's going to be useful for studying human disease as well. Now, as a physician I was always interested in eventually applying what I was learning to human systems. And more and more as I get on in my career I'm looking for opportunities to take the vast amount of knowledge we have, for example, about drug resistance and actually use that to improve treatment of cancer. But early on it was curiosity driven science. There was a strong culture of choosing interesting problems and pursuing them and learning as much as you possibly could about biological systems with very powerful experimental techniques.

Yeah, I don't remember any discussions about budget. I think Marty himself had a certain kind of frugality both in the simplicity of the experiments that he designed and in the desire to, you know, either expand his -- the lack of desire to expand his laboratory or to engage in very expensive, you know, high throughput kinds of studies. So, I -- from the beginning I think I had a sense that you could do really good science without a lot of money and there was never any discussion about, you know, we had the equipment we needed and we got the supplies we needed. And I don't remember at any point any discussion except the occasional comment by people about the fact that we had a central unit that washed all of our glassware so it got sent off and it came back washed and sterilized -- flasks and so on. Now that unit has since gone by the boards because it was too expensive to maintain and people moved over to more disposables and so on and so forth. But we used to talk about NIH experiments -- we had a central media unit. I mean, there were a lot of amenities that totally took all the grunge work out of doing science. And I think on occasion we appreciated that but after a while you'd come to expect it.

And you know, I've heard to this day that one of the main complaints that the fellows who are here now have is not that they don't have a wonderful experience and they don't have an opportunity to do great science but when they leave here they're somewhat spoiled and incapable of dealing with the real world restrictions on what may be available in the laboratory to support their research.

Yeah. So, I should probably say something about the leadership at NIAMS because the reason that the intramural program was so dedicated to basic science was that Ed Rall was the scientific director and later became the equivalent of the deputy director for intramural research for the whole NIH. So he was a really strong proponent of high quality basic -- very rigorous basic science. And I think that set a tone for the institute and eventually for the intramural program at the NIH when he became director. So, he was responsible. It was -- it was at the level of the scientific director that recruitments were made into the research associate program. In fact, I remember at one point it was a scary moment after I had been accepted into the public health service which is a uniform service and a substitute for military service -- this was remember the time of the doctor's draft. I

got a note from my draft board saying I was supposed to report for duty on such and such a date and I was supposed to go to Fort Mead for my physical and various other things. And I, you know, I didn't know what to do because I had already been accepted into the public health service. So I called Ed Rall and Ed said, "Don't worry, I'll take care of it." And somehow it went away. So I think he called whoever the commanding officer was at Fort Mead and said, "We got this one already, he's not yours." But it was a very interesting time. There was a lot of anxiety of young people about having to go off to a very unpopular war at a time when their intellectual lives were just starting to take off.

The public health service was part of the Department of Health and Human Services or Health Education and Welfare, HEW, at the time when we were here. And it included a lot of the, in fact, the laws that govern NIH are the public health service acts. But in the public health service was a branch of officers called commissioned officers6 in the public health service. And it was a uniformed service. You've seen people wearing their uniforms with Navy equivalent rank. So I came in as a Lieutenant Commander which is an -- I guess an 03. And maybe 04 -- no, 03, it was an 03 -- which is a military rating system. And although we didn't wear uniforms we would occasionally have to have a uniform for some official function. And so there was usually one uniform that got passed around and one size did not fit all but one size was worn by all. So we had -- there were pictures with people who were totally floating in their uniforms and others where they looked like they're wearing their confirmation suits [laughs]. So, they're -- so the core was a uniformed part of the commissioned officers. It was -- it had a pay scale, which was at various times better or worse than the civil service pay scale. When I came in it was somewhat better than the equivalent postdoctoral fellow would get. And it was an opportunity to do the equivalent of public service for military service and it was a three-year stint for me. And then after that period I did go back to Harvard. I wanted to finish my residency. At that point Eugene Braunwald who had been an NIH branch chief. He was the head of the cardiology branch I guess in NHLBI, then the National Heart Institute, NHI. But he was back then as Chief of Medicine at the Peter Dent Brigham Hospital where I had done my internship and he was anxious to get me back. He said, "You've had three years of research experience. You don't have to do your junior residency. So if you do a senior residency I will see to it that you're eligible to take your boards in medicine. Normally it's a three-year requirement. So I was able to do the three years in two years. I came back as a senior resident and, you know, pretty much stepped back onto the wards. And then because of my long-term association with Bert Vallee, who had been my mentor as a medical student who was at that point very interested in tumor angiogenesis -- he recruited me to come and work on a project that he had started with Judah Folkman. And Judah was working on the biology and Bert was interested in identifying the actual factor or factors that were responsible for tumor angiogenesis. So, for a year I had an appointment in the Department of Anatomy in Harvard, which later became Mark Kircshner's Department of Cell Biology. And I tried to set up systems to study tumor angiogenesis. That research led to sort of other finding unrelated to tumor angiogenesis. So my strategy was to take tumors that we knew made factors that caused growth of blood vessels and I noted that there were four or five major factors secreted by these cells and I started to purify them. And one of them was expressed at high levels in the tumor cells but not in the equivalent cells that were not malignantly transformed. And it was the first -- it was the first factor isolated from the extra6 cellular secretions of tumors that didn't have angiogenesis activity. So I ended up studying what turned out to be Cathespin L, which was a -- I called it major excreted protein. And Cathespin L is a cysteine proteinase. It's an acid cysteine proteinase which normally

is present in high levels in the lysosome but many tumors secrete it. And, we still don't know exactly what it does. So I studied, that was kind of a side thing for a while. And then after a year as the -- as an assistant professor of anatomy at Harvard Medical school and with all the responsibilities of teaching medical students and working in the laboratory, Susan at that point was at MIT working with David Bottstein. So, she'd done a postdoctoral fellowship with Max Gottesman in the Cancer Institute, in Ira Pastan's lab. And then had done -- when I went back to Harvard -- we, I should point out we alternated primary choice of where to be. So, I chose NIH. She chose to go back to Harvard. And then we decided we needed -- she had a great job with David but it wasn't a faculty level position. Whereas when we were recruited back to NIH we were offered two PI positions basically -- what we now call Senior Investigator positions. So, for me it was quite early in my career. I mean, I had a done a few years of research at the NIH basically. I was like a senior post doc but I was being offered a tenured position at the NIH too good to turn down.

So, I'm pretty sure that Ira Pastan and Al Rabson had a huge role to play. So, Ira knew Susan extremely well because it was in Ira's lab that Susan had done her postdoctoral fellowship and he was very anxious to recruit her back. He recognized, you know the brilliant scientist that she is and he was at that point building his laboratory. And he was building it to the extent that the laboratory of biology -- he was the laboratory of molecular biology at NCI. The laboratory of biology was being closed down and Al Rabson who was the scientific director of our division had asked Ira to encompass that laboratory of biology in his division. So there was space there to recruit other people and Susan said, "What about my husband?" And he said, "Oh, bring him along." [laughs] So, I knew Ira reasonably well and I certainly knew Al reasonably well and Al was enormously generous in offering me a position as well. Now, as I've pointed out to NCI that was before all the rules that I put into place about the strict requirements for searches for people who are coming in to permanent positions, all the complicated tenure requirements that we've put into place -- whether or not I would have been recruited to come to the NIH in the current circumstances I can't say. But I was delighted that I was and Susan and I both had permanent positions at the NIH laboratories. We both had labs in the laboratory of molecular biology, which was Ira's lab. And my job was to establish a research program. So, I spent a little bit of time developing -- I mentioned somatic cell genetics so I was able to get actually not so much from Ted Puck but from Lou Siminovitch who was also a very senior somatic cell geneticist. There weren't that many people -- in Canada, at the University of Toronto. And Lou was extremely generous in providing all kinds of cell lines and mutant cell lines. And we began to study the growth of mutant Chinese hamster ovary cells. One of the very first projects because of the interest that Ira had in cyclic AMP function was to say could we get mutants that are affected in the pathway for cyclocane p effects on cultured cells. So if you add cyclocane p analogs to CHO cells they stop growing and they change shape. So, it was pretty straightforward since they stopped growing to select mutants that continued to grow. And all of them turned out to be mutants and cyclic AMP dependent protein kinase so it's the first genetic demonstration that the kinase which was known to be activated by cyclic AMP was actually responsible in the pathway for cyclic AMP activation of various functions. And we were able to look at the mutants and figure out what cyclic AMP was doing that was dependent on the Kinase. And actually, some of those mutants have turned out to be prescient because a few of them have reappeared in the type of mutant -- either the regulatory or the catalogs [unintelligible]. Have really appeared in human genetics as the cause of some diseases of cell growth. Like certain kind of tumors. So it turned out that those were good

models but 30 years later for human tumors. So, we were emboldened by isolating these mutants and at that point I think I was influenced by Bruce Chabner who was the scientific director. So Al Rabson was the director of our program, the Division of Cancer, Biology, and Diagnosis, DCBD and then there was the Division of Cancer and Chemotherapy that Bruce Chabner was scientific director of. And Bruce and I used to chat occasionally and Bruce said "You know, we treat patients who have cancer with all kinds of drugs. Sometimes we get really good responses, sometimes they don't respond at all but when we get really good responses all too often the tumors recur, they relapse. And the end stage for many cancer patients is just tumors that are resistant to everything. We call that multi drug resistance. You know, do you think you'd be interested in studying why tumors become multi drug resistant. And you know, you have a system to analyze that." So, I began -- at that point I had my first postdoctoral fellow, a guy named Fernando Cabral who was doing his second post doc. He had done his first post doc looking at mitochondrial biochemistry basically. And he and I sort of set up working together sort of side by side. We were about the same age although he was a post doc and I was a senior investigator. And we worked together on developing system that in which we could study the basis of resistance to anti-cancer drugs initially using CHO cells. And in simple terms we got two kinds of mutants. We got mutants that were specifically resistant to the drug. So if we used an anti-microtubule drug like Vinblastine and Vincristine, which are used to treat cancer we got mutants that were in tubule and we could demonstrate -- he was an expert in running 2D gels. You could see the change in mobility in the mutant tubulin because the changes in the amino acid composition resulting from mutations. Most of these were mutations that affected the stability of microtubules. So for example, if the drug was a drug like Vincristine which depolymerizes the microtubules so a change in tubulin that made the tubule more stable would make the cells resistant to the anti-microgen. So you weren't knocking out tubule. What you were doing was changing its relative stability as a polymer. If you used a drug like Taxol which works by stabilizing microtubules the mutants we got had relatively less stable tubulin. In fact, under some conditions they were actually Taxol dependent because the microtubules there were so unstable they needed a little bit of Taxol to grow. So that -- there's a whole bunch of papers and interesting story there. And the other class of mutants we got, the ones that weren't target mutants, were multi drug resistant general mutants. Now, at this point there was a -- some work had been published by Victor Ling on a similar class of mutants in CHO cells that he had been studying and he had found using simply biochemical techniques that there was a protein on the surface of those cells which he called p glycoprotein which he thought was affecting the permeability of the drugs into the -- in to the cells. And the reason he thought that was when he looked at the cells and then took a radioactive drug the resistant cells accumulated less of the drug. So that was kind of a background but we didn't have any idea when we started isolating mutants what we would get. And so we used an agnostic technique to try to clone the gene. And this story is actually quite interesting and it's a story that I used to point out why it's important for scientists to go to scientific meetings. It is really important. So we had -- maybe take one step back. So I was in Ira's lab, and Ira and I were sort of interested in working together on some of these multi-drug resistant mutants with resistance to cancer cells. And we decided that in order for this to be useful we would work with a system which was a human system and this was—the Chinese hamsters were fine, but all the free agents we'd have to develop would be hamster-specific. We wanted human-specific ones. And in fact I have someplace a strategic plan, believe it or not, I wrote a strategic plan in 1983 I think, describing how we would go about identifying the gene responsible for drug resistance. The first step was to isolate mutants. The second step was to use the mutants to figure out what was mutant in those cells so that we could isolate the gene,

transfer the gene, demonstrate it was responsible for multi drug resistance and so on. And we had at that point a wonderful post-doctoral fellow from Japan, Shinichi Akiyama, and Akiyama set about using ten or so different cultured cell lines to find cell lines that grew quickly, that were sensitive to anti-cancer drugs, and in which we could easily isolate resistant mutants. And after a huge amount of work he came up with a cell line, which we got from ATCC that was called KB. And ATCC said KB is a nasopharyngeal carcinoma. We didn't really care; we had a cell line to work with. Subsequently we found out based on our own mapping and so on that we had HeLa cells like everyone else in that period. HeLa cells will quickly overgrow any cells in culture and ATCC was providing what they called KBs but they were really HeLa cells. So no surprise we had rediscovered that HeLa cells were great for studying genetics and tissue culture. They grow quickly; they're sensitive to drugs. And he isolated -- our strategy was to isolate a series of mutants of increasing resistance hoping to amplify the gene that was responsible for the resistance and at that point Bob Shimpkey had done his pioneering work on methotrexate resistance to hydrofoil reductase amplification so we knew this was a possible mechanism of resistance in cultured cells. And so we were very -- really quite careful in isolating single cells and single steps, characterizing their resistance patterns, pulling out the ones that were cross-resistant to multiple drugs and then reselecting for higher levels of resistance looking for the same pattern of drug resistance. Because if they were a single gene responsible it should be amplified. And we got four or five different steps. We got pretty highly resistant cell lines, we had series of cells and we were about to embark on isolating the amplified gene. I went to a Gordon conference and I presented my work at a poster session there. And I was standing next to another poster session by a man named Igor Aronson who was a Russian [unintelligible] who was a post doc in Alex Warshawsky's lab at MIT. And Igor is an incredibly creative, technically proficient scientist and his poster was about cloning amplified genes. And he had developed a technique and he wasn't thinking about multi drug resistance. He was just, you know, figuring out how to close these genes. So the technique involved taking DNA from a series of cells, some of which had amplified DNA and some of which did not, running them out on a gel, digesting the DNA within the gel so that you have all the different restriction fragments, and then reannealing -- rapidly reannealing. And you would expect if you rapidly reannealed DNA in a gel that only those pieces that are close to each other -- in other words, the same size -- and at high copy number would be able to reanneal. Single copy genes -- the conditions were set so single copy genes would not reanneal. And these were end label fragments, the fragments had been end labeled so it was just a matter of doing an autoradiogram. So you digest with the restriction enzyme, you reanneal and you take DNAs that digest single-stranded DNA and the only things that remain are the reannealed double-stranded amplified genes. And you get -- so in a normal cell where there are lots of sequences amplified you see maybe 20 different bands on the gel. And in our resistant cells we found the 20 that were present in most human DNA plus extra ones. And because we had the series of cell lines we could see which of those extra bands were present in a lot of drug resistant drug lines. You cut them out of the gel. You clone them and we recreated a full length CNDA and we sequenced it. All of which was not easy in 19 -- we're talking about 1985 now roughly. And all of that information led to the cloning of a full length CNDA. At that point we had another great Japanese scientist at the lab, Kazumitsu Ueda, and Ueda showed that you could recreate a vector with the full length CDNA. You could express it in cells and they became multi drug resistant and they expressed a protein on their surface, which we later showed in a collaborative piece of work with Victor Ling, was o-glycoprotein. So, we had by a totally different technique -- now in science when two different people coming from different ways of thinking about a problem arrived at the same conclusion

people actually get excited. So this is -- and that paper, which showed that p-glycoprotein, was the MDR1 gene that we had cloned was actually a BBRC paper. But I think a really important paper because it allowed the field to move forward. It had diverged in a different direction. And then we had a period from about '85 to about '93 -- about eight years. So the sequence paper was published Igor Aronson in Cell. The paper showing that you could express he full length CDNA was in the proceedings of the National Academy of Sciences. We had a couple of science papers along the way studying the expression of how expression of the MDR1 gene or PGP gene comes about. And we were interested in the physiology of this gene. You know, why was it expressed? Where was it coming from and so on? So we worked with a colleague of Ira's named Mark Willingham who was a wonderful histo-chemist and microscopist who showed that the gene we had cloned from these drug resistant cell lines was expressed in certain epithelial cells in the human body. So it was expressed in the kidney, in the proximal tubule cells in the liver and biliary epithelial cells in the GI tract and the cells lining -- all these were barrier areas where you could plausibly say the function of the protein was to either keep things from being absorbed into the GI tract or pump them into the bile or into the urine and that's later been shown to be the case. But it was also expressed in the brain at the blood brain barrier and that's turned out to be one of the more interesting aspects of it. So, those studies I think led to our understanding of the normal physiology of this protein. It is a barrier protein. It's a -- it turns out to be an extremely important protein that handles a lot of drugs that are able to be excreted -- kept out of the body or excreted from the human body. So these are things you eat in your diet that are present in microorganisms or plant products that would otherwise be very toxic except for this protein.

So people kept asking us what the normal function of the protein was. And initially we said, "We're not sure what its normal function is." Later on Pete Borst was able to create transgenic knock out mice and they were fine until you fed them a toxic compound. And the toxicity was usually neurotoxicity because many compounds would get into the brain. So it turned out, I think from the mouse studies and -- that probably the normal function is exactly that -- to protect us from the xenobiotics that we're in a sea of these things all the time. So the ancestry of this protein goes back a long, long way. Every organism has related proteins and I think it dates back to when cells started putting membranes around them. The danger of having microorganisms create toxic products was met by having this pump system that pumps things out of cells. And the whole story about how we unravel the biochemistry demonstrated that it -- that system was a transporter. That work was done with my good colleague Suresh Ambudkar who we were able to reconstitute purified protein and demonstrate it could transport the drugs. And the continuing story of how this works, how a single protein can recognize literally hundreds of different drugs, which is antithetical to the one enzyme, one drug -- one substrate process is fascinating. And we're just beginning to get -- we and others are trying to get higher resolution crystal structures to understand the interaction between substrate and protein and how pumping is initiated and so on. One of the -- along the way one of the ideas that we came up with which I think has stood the test of time was that this is not a traditional trans-membrane pump system. What it is a system that is probably recognizing the substrates, which are by and large hydrophobic -- in other words, lipid soluble. There were -- it's recognizing them in the plasma membrane. So as they get into the cell the pump is there, it sucks up these6 hydrophobic compounds and spits them out and keeps them from accumulating in the cell. So it's -- the reason it has such broad lack of specificity is its advantage is not thermodynamic but it's kinetic. So at equilibrium it couldn't possibly recognize with enough affinity 1000 different compounds. But since it's seeing them at high concentration as they enter

the cell it's able to deal with them and pump them out. So, it's an interesting kind of side light to the biochemistry.

So, in terms of clinical development I think our disappointment has been that although PGP is expressed in cancer cells it is a -- it certainly is one of the causes of drug resistance there have been a lot of development in terms of developing compounds that inhibit its function because it's actually relatively easy to get inhibitors because it has so many substrates so anything which is a non-toxic substrate6 becomes an inhibitor basically, because it, you know, it basically is competitive with whatever toxic compound you run again into the cell. So it's easy to inhibit it. But when you do that in people -- first of all, the inhibitors tend to be pretty toxic for obvious reasons. But also, other mechanisms of resistance in the cells that express it are -- become obvious very quickly. So you may get a transient response but then the tumors will become resistant again. So drug resistance is a very complicated phenomenon and clearly a single gene responsible for resistance is a dream, which has turned out not to be the case. But, it turns out to be really important in drug metabolism because so many different compounds are affected in terms of their uptake, their excretion, their distribution in the body. And in fact, I think probably the biggest impact pharmacologically has been that virtually every pharmaceutical company that develops a new drug has to characterize its ability to be transported by p-glycoprotein. The FDA is now requiring that and it's turned out to be enormously important information for figuring out drug-drug interactions. So sometimes one drug affects the accessibility of another drug and it can happen at the metabolism level or at the transport level and PGP is an important transporter. Now, the other kind of sidelight to all of this is that p-glycoprotein or MBR1 was the first of what are now known to be 48 human ABC transporters, ATP dependent transporters. And that was -- a number of people contributed eight or nine years after our initial discovery to the finding that there were other members of this family and eventually when the human genome was sequenced there were 48 members. About a third of them or a quarter of them are probably involved in drug transport. Now all multi drug transporter. Some of them incidentally transport drugs that are related to their normal substrates. The others have very specific functions about half of them are the known cause of Mendelian genetic disorders. And others will be, I think, in time as we hear about them. So each of them seems to have an essential kind of function, not essential for life necessarily but essential to have normal -- to not be diseased.