CHEMICAL HERITAGE FOUNDATION

MAKOTO KURO-O

The Pew Scholars Program in the Biomedical Sciences

Transcript of an Interview
Conducted by

Robin Mejia

at

University of Texas Southwestern Medical Center
Dallas, Texas

on

6, 7, and 8 March 2006

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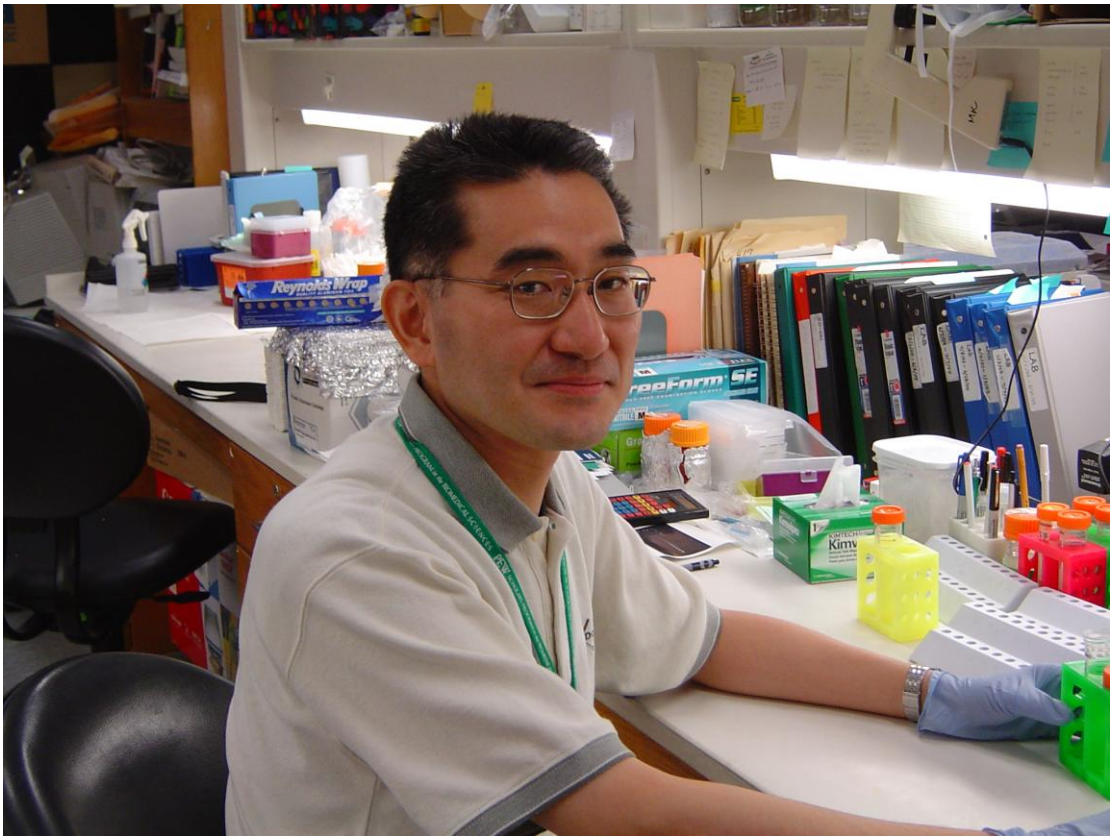

Makoto Kuro-o

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Interviewee's Name

January 6, 2022

Date

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315 Chestnut Street
Philadelphia, Pennsylvania 19106

MAKOTO KURO-O

1960 Born in Tochigi, Japan, on 7 January

Education

1985 M.D., University of Tokyo
1991 Ph.D., University of Tokyo

Professional Experience

Tokyo University Hospital, Tokyo, Japan
1985-1986 Intern

Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan
1986-1987 Intern
1987-1988 Resident in Internal Medicine (Cardiology)

University of Tokyo
1988-1991 Clinical Fellow**,** the 3rd Department of Internal Medicine

National Institute of Neuroscience, NCNP, Tokyo, Japan
1991-1994 Postdoctoral Fellow**,** Division of Molecular Genetics
1994-1998 Domestic Research Fellow**,** Division of Molecular Genetics

The University of Texas Southwestern Medical Center at Dallas
1998-2006 Assistant Professor of Pathology
Southwestern Medical Foundation Scholar in Biomedical Research
2006-present Associate Professor of Pathology

Honors

1992 Young Investigator's Award, Japanese Circulation Society
1997 Irvine H. Page Arteriosclerosis Research Awards for Young Investigators (Finalist), American Heart Association
1998 Erwin von Bälz Preis, Boehringer Ingelheim
1999 President's Research Council Distinguished Young Researcher Award, UT Southwestern
1999-2003 Pew Scholar in the Biomedical Sciences

Selected Publications

Yazaki Y, Tsuchimochi H, Kuro-o M, Kurabayashi M, Isobe M, Ueda S, Nagai R, Takaku F. Distribution of myosin isozymes in human atrial and ventricular myocardium: comparison in normal and overloaded heart. *Eur. Heart J.* 5: 103-110, 1984.

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ABSTRACT

Makoto Kuro-o grew up in Tokyo, the younger of two children. His father was an engineer, providing air conditioning systems for large structures like the Tokyo train stations. His mother went to college but did not work after she married. At an early age Kuro-o decided he liked science. He attended the local elementary and junior high schools, but a national high school. His high school chemistry and physics teachers were enthusiastic about their subjects and helpful to Kuro-o. At this point he contemplated becoming a doctor; he talks about the higher education system in Japan, his experience getting into medical school, his parents' expectations. He entered medical school at University of Tokyo. Because his father had a heart attack during Kuro-o's second year in medical school, Kuro-o became interested in cardiology and describes his first basic laboratory experience. He did his PhD while spending at least half of his time seeing patients. He met Ryozo Nagai and joined his lab at Tokyo University. Here he discusses his funding; Nagai's research interests; publishing articles; and his postdoctoral work in genetics at the National Institute of Neuroscience of Japan on the age-suppressor gene in mice. During his last year of internship Kuro-o met and married his wife.

Kuro-o then accepted a position at the University of Texas Southwestern Medical Center. He talks about his move to the United States; setting up his laboratory; funding in general and specifically the impact of the Pew Scholars Program in the Biomedical Sciences on his work; his lab management style; his teaching responsibilities; and his research on the age-suppressor gene. Next Kuro-o discusses a little more of his research on the age-suppressor gene, his current research on the anti-aging protein and renal disease, and practical applications of his research.

Kuro-o then moves on to talk about his future research on the functions of the Klotho protein and about his collaborations, tenure at University of Texas Southwestern Medical Center, his administrative duties, his role in the lab, the running of his laboratory, the process of writing journal articles, and patents. He also describes a typical work day. The interview concludes with Kuro-o's comments on collaborations in science, serendipity in his work, gender and ethnic issues in science, his first impressions of the United States, and a comparison of science in Japan and the United States.

UCLA INTERVIEW HISTORY

INTERVIEWER:

Robin Mejia, Interviewer, UCLA Oral History Program; B.A., Biology, University of California, Santa Cruz, 1997

TIME AND SETTING OF INTERVIEW:

Place: Makoto Kuro-o's office at UT Southwestern.

Date: March 6, 7, 8, 2006.

Total number of recorded hours: 4.5

Persons present during interview: Mejia and Kuro-o.

CONDUCT OF INTERVIEW:

This interview is one in a series with Pew Scholars in the Biomedical Sciences conducted by the UCLA Oral History Program in conjunction with the Pew Charitable Trusts' Pew Scholars in the Biomedical Sciences Oral History and Archives Project. The project has been designed to document the backgrounds, education, and research of biomedical scientists awarded four-year Pew scholarships since 1988.

To provide an overall framework for project interviews, the director of the UCLA Oral History Program and three UCLA faculty project consultants developed a topic outline. In preparing for this interview, Mejia corresponded with Kuro-o by email and talked by phone to obtain background material, including Kuro-o's CV, and to schedule the interview. Mejia also obtained and read copies of Kuro-o's published his articles, reviewed his descriptions of his work on his website, and reviewed background information on the institutions at which he has worked and the countries in which he has lived.

ORIGINAL EDITING

Carol Squires edited the interview. She edited for punctuation, paragraphing, and spelling, and verified proper names. Words and phrases inserted by the editor have been bracketed.

Kuro-o reviewed the transcript. He verified proper names and made a number of corrections and additions.

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INTERVIEWEE: **Makoto Kuro-o**

INTERVIEWER: **Robin Mejia**

LOCATION: **University of Texas Southwestern Medical Center**
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MEJIA: This is Robin Mejia here with Makato Kuro-o in his office at U.T. [University of Texas] Southwestern Medical Center on March 6 [2006] for the first day of the Pew [Scholars Program in the Biomedical Sciences] oral history interview.

Hi.

KURO-O: Thank you. Hi. How are you?

MEJIA: Good. It looks like we're picking up just fine here. So I thought we would start this at the beginning. Could you tell me where you were born and where your parents and family are from?

KURO-O: Okay. I was born in Tokyo in 1960. My parents [Masahiko Kuro-o, Nobuko Kuro-o] are in Japan, and my wife is Kumiko Kuro-o. She was born in 1961 in Tokyo, too.

MEJIA: Can you just tell me a little bit about your parents, what they were like, what they did?

KURO-O: Okay. My father [Masahiko Kuro-o] was an engineer working on designing some air-conditioning systems or something like that. He graduated from Tokyo Industrial University. It's also in Tokyo, but he is originally from a little bit north, northern part of Japan. My mother [Nobuko Kuro-o] was also from there. My father is currently—let me see—eighty years old, and my mother is five years younger, so she is seventy-five, if I remember correctly.

I have one sister [Atsuko Rakuman], an older sister. She is four years older than me. I am now forty-six, so she should be fifty. She got married and has two children, a boy [Satoshi Rakuman] and a girl [Kaori Rakuman]. They are not a boy and a girl, actually; they are in college right now, so they are pretty much grown up. I have no kids. That's my family.

MEJIA: And did your sister stay in Japan?

KURO-O: Yes, they live in Japan. They are very close to our parents.

MEJIA: Are your parents both from the same community? Did they move to Tokyo?

KURO-O: Yes.

MEJIA: What brought them to Tokyo?

KURO-O: My father got a job in Tokyo after graduating from the university. Actually, the university was in Tokyo. When he graduated high school in his town, then he moved to Tokyo to go to university. So since then, he stayed in Tokyo and got a job in Tokyo. But when he got married, he just went back to his hometown and he got married with my mother there. After they got married, they both, again, came back to Tokyo. It's not very far, just a bus [ride] down to Tokyo. It's just two hours by train.

MEJIA: Probably better work opportunities?

KURO-O: Yes. Yes, better work opportunities in Tokyo.

MEJIA: They had you after they were both in [Tokyo]?

KURO-O: Yes.

MEJIA: So you were born in Tokyo after they had both ended up there?

KURO-O: Yes. Yes. Right. Yes. I think I was born six years after they married, maybe. Yes, something like that.

MEJIA: Okay. What level of education did your mom have?

KURO-O: My mother just had college. The system was very different at that time. It was before World War II, but around World War II. She graduated college, and she actually had never worked. After graduation, she got married with my father, and then she never worked, just doing the housewife, housekeeping. That's rather traditional style in Japan, actually.

MEJIA: I think your microphone's gone hiding there.

KURO-O: Oh, yes. Okay. Maybe this is not good. Better?

MEJIA: That looks great.

KURO-O: Yes. Okay.

MEJIA: And to be an engineer, was that an advanced degree or a college degree?

KURO-O: Not college. He graduated from a university program. I'm not so familiar with that. That is a different system, so it's probably similar to the graduation of university for a B.S. [bachelor of science degree] He didn't go to graduate school, but he just graduated university. Yes.

MEJIA: Can you tell me just a little bit about your neighborhood?

KURO-O: In Japan?

MEJIA: In Japan, yes.

KURO-O: Okay. Well, I lived in the north central part of Tokyo, a little bit urban area of Tokyo. What do you mean by neighborhood? Just what kind of people were around?

MEJIA: Did you have friends in your neighborhood? Did you go to school close by?

KURO-O: Sure. Yes, sure. I went to elementary school and junior high school in the local junior high school and elementary school. Many friends at that time were also living very close

by, so it was a local community. Some very clever boys and girls can go to the more high-ranked school, but it's very expensive. My parents are not very rich, just average income, and so I just went to the local schools. After graduating junior high school, I didn't in high school. Anyway, we just stuck in the local community.

A good thing in Japan is no huge difference in income. In the [United] States, rich people are very rich, and poor people are sometimes very poor. But the good thing in Japan is there's not very much difference in the income, so the way of life is very similar. That was a good thing for me, in a way. That was the community I was in growing up. Nothing in particular, actually.

MEJIA: So what you're saying is, your friends in the area, even though your families were different, it wasn't the same differences.

KURO-O: Right.

MEJIA: Did you have any particular interests in school at that time or just everything, like a lot of kids are?

KURO-O: Well, yes, maybe I'm not very different from others, but I'm interested in science rather than literature. Yes, science or chemistry or physics or something like that. I don't know why. My father was an engineer, so that might have had some influence.

MEJIA: Did you talk about engineering with him, or his work?

KURO-O: No, not very much. I really respected his work, because he didn't design small air-conditioners. Actually, his major was to build up a building, or storehouse to keep the imported meat, or some big, huge structure. His work includes the air-conditioning system in the Tokyo Station. Of course, it's only a part of it, but he is very proud of that. The good thing for such kind of work is you can actually look at what he did. So that impressed me a lot. He didn't talk much about science, but maybe I thought, oh, my father is great. [laughs] I had a little bit of the favor of science or chemistry, physics, or such kind of area.

MEJIA: Even before high school?

KURO-O: Right. I think so. I think so.

MEJIA: Any particular memorable classes or teachers or projects?

KURO-O: Yes. Is it okay if I talk about high school?

MEJIA: Sure.

KURO-O: Okay. In high school, I had two teachers, very impressive for me. One taught physics [Mr. Takahashi] and the other taught chemistry [Mr. Yoneyama]. Actually, I don't remember very well about what he taught me, but I was impressed with their personalities. They were really strict, and many students were afraid of them, but they were really, in a sense, very kind if I had any questions. If I asked any questions, they were very enthusiastic about answering the questions. I actually forgot what I asked them, but they were really enthusiastic teachers.

Actually, the teacher I was most impressed with in my early education, I met him in —what do you say?—the preparation school. I spent one year after graduating high school until I got into Tokyo University. There is one year blank because in Japan, the entrance examination to the university is very difficult sometimes. I was not accepted in any school just after I graduated high school. So I had to prepare another year, study hard and get a high score in [the] entrance examination.

During that period, the students usually go to some school that teaches how to increase the score. It's kind of a technical school, but people usually don't expect so much about the teaching, about the personality. But one of the teachers I met there was, again, a physics teacher [Mr. Sakama], and he was actually a professor at another university, the University of Saitama in Japan, and he spent some time teaching dropped-out students like me. His lectures were really excellent. He taught us—I don't know how to say it in English—it was like Newton's classical [physics]. What do they say?

MEJIA: Classical physics?

KURO-O: Classical physics, yes. At that time I had a little bit of confusion about physics and how to understand physics, but he introduced mathematics to explain how classical physics works. At first I couldn't understand anything, and in the meantime, his lectures were very nice. I finally understood everything. So it was really my first encounter with scientific thinking, I think. This was actually a very classical formula. Of course, I learned this when I was in high school: first rule, second rule, third rule, something like that. But I just memorized it [the rules], and I didn't know where this [rule] came from; I didn't know about the principle. I didn't know the principle at all. But he used mathematics to explain this, and he explained everything just on paper without any experiment. During the lecture, I understood that science is built up from very

simple principles, and everything can be deducible from very few principles. To find those few principles is very difficult, maybe, but I thought it's a very beautiful system, and I was very impressed about how beautiful the physics system is. Probably that is the most direct reason why I wanted to devote myself to science. It's really impressive.

Usually, in preparatory school, nobody expects that. They just expect a technical thing: how to answer these kinds of questions or programs or something like that. Usually it's not a very fruitful year, just preparing to go to the university. People expect such things in the university, actually. But, yes, I was lucky. So that was the most impressive teacher for me.

MEJIA: I'll actually want to come back to that, because it sounds like it was probably an important period for you. But if we could step back for just a minute, you said you went to the local grade school and junior high or equivalent.

KURO-O: Yes.

MEJIA: So what happened next? How did you end up deciding, or your parents deciding, or how did you end up going to a different high school? Can you talk about that?

KURO-O: There are several different high schools we can choose from, and of course there are several local high schools. There are some local high schools that are really good schools, so many students just go there. But there is another area; it's a private high school. There are many good private high schools, but they are usually far from where we live, so you have to spend one hour to commute there. Another way is to go to not a local but kind of national school, something like that. So there are three ways: a local high school, a private high school, and national high school. It's run by the government. There are three ways.

I went to the national school, partly because it's cheap. Actually, it is really difficult to get into. The entrance examination is really difficult. So maybe only the top 1 percent of students can enter this school. I was really lucky; I passed the entrance examination. So that was really to my advantage for the education. Of course, the teachers were really nice. As I said, I met two good high-school teachers, the physics and chemistry, there. So that school was very nice, and many students in the Tokyo area come to this school.

MEJIA: Two quick questions. Is that somehow associated with the university, that school?

KURO-O: No, it's not associated with the university. But, of course, some private schools have some association with the university. But in my high school, there was no particular association.

MEJIA: At this point, I guess, how important was education in your family at home? Was that part of a driver, or was this more of a personal thing from inside you, or a mix?

KURO-O: My father graduated university, so naturally they expected me to graduate from university. Actually, many Japanese students at that time, maybe more than 50 percent of the high-school students, went to the university. Recently the rate is much higher, maybe 70 percent. You might not believe that, but in Japan, university education is relatively cheap. So many high-school students can go to the university. But at that time, the rate was probably around 50 percent or so. But anyway, my father graduated the university, so I thought of course I should go to the university. I didn't think about not getting higher education.

When I graduated high school, I began to think about being a medical doctor, probably because my parents suggested it to me, not forced me. But a doctor was a good occupation. So maybe I was gradually educated by them to be a medical doctor. But I also think, of course, a medical doctor is a very important occupation, and it's a very respectable occupation. At that time, I really wanted to be a hard-core clinician.

MEJIA: A hard-core what?

KURO-O: Clinical doctor, so look at patients and cure disease. At that time, I didn't know anything about medicine, but I wanted to be a good doctor.

MEJIA: Did you think about research at all at that point?

KURO-O: No, not at all. For a young high-school graduate student, when I say doctor, that meant clinical doctor. No idea about research and how research is going on, I had no idea at that time. I wanted to be a medical doctor, so I entered medical school in Tokyo University.

MEJIA: So you applied once out of high school and again out of the preparatory school?

KURO-O: Yes. I spent one year, and then the next year I was successful. It's a really difficult entrance exam, especially medical school in Tokyo University. It's really tough. In the United States, I heard that entrance is not so difficult, but graduation, to graduate a school is really tough, especially a good university. But in Japan, it's different. To get into a good university is really tough. It's very severe competition at the entrance. But once you enter school, you don't have any difficulty graduating if you just study, obviously.

MEJIA: I know I asked you to clarify this earlier, but for the recording, too, can you explain how the system works there, which is a little bit different than in the U.S. [United States]?

KURO-O: In the university. Okay.

MEJIA: Yes. Because you already knew out of high school that you were applying to a medical program, so could you explain how that works?

KURO-O: Yes. Once we entered the university, we spent two years getting some basic education about any science, like biology, of course, and any area of science, like philosophy or statistics or mathematics, any kind of science, not very in depth, but just getting an idea about what the, say, philosophy looks like or something like that. We spent two years learning foreign languages there.

In that elementary two years, all students were in the same class. There were no medical students. Everybody received the same lecture, the same education. But after two years, we were divided into specialties. In my case, of course, we went to the Department of Medicine, and the special education started: anatomy, physiology, biochemistry, the basic science first. Then we spent two years for that, and then another two years for clinical stuff like internal medicine, surgery. We rotated through the hospital, university hospital, and just looked at patients or just talked with patients. It's probably very similar in the United States. So two years of basic education, two years of basic research education in medicine, and two years for clinical medicine, and then we graduated after six years of education.

MEJIA: And those first two years, when you say you're all together, is that everybody who's going to go into the medical school, or is that everybody at Tokyo University? Because the university looked huge when I found it on the website.

KURO-O: Yes, right. Right. Exactly. There are probably more than three thousand or four thousand students in a class, and only ninety people are for medical school. Let's say one thousand students are for other areas. Some are law school, some are economics, or something like that.

So the high-school students apply for each area first. So if you want to go to law school in the future, the high school student will apply for the law-school course, but in the initial two years, they are all mixed, that is, educated in the same way. But after that, they go to the law school they applied for at the entrance examination. So it might be a little bit different system, maybe.

MEJIA: So it's two years of essentially general education?

KURO-O: Right. Right. Is it like that in the United States?

MEJIA: No. It varies. Most universities have some what they call either comprehensive or general education requirements, but you wouldn't necessarily all be taking them together. By the end of your four years you have to have finished this much general stuff in addition to whatever you choose for your major, and then your major will set its own requirements.

KURO-O: I see. Yes. There is some flexibility, but essentially we go through these steps. Especially in medical school, there's no room to choose. All the classes are mandatory. But anyway, it's really a long education, six years.

MEJIA: Did you continue to live at home, or did you move for school?

KURO-O: I commuted from my house. It's just one hour from my house. So I stayed with my parents. I just went to the university from my parents' house. I got independent from my parents after I graduated from medical school. So it was cheap for my parents. I didn't eat up their income. The good thing is, at the University of Tokyo, tuition is very cheap, actually. It's only maybe one or two thousand dollars for a year, everything, including everything. Of course, private school is really expensive. So maybe my parents couldn't afford that. There are many good private medical schools, but tuition is maybe a hundred times more expensive, really different. So maybe only very rich people, like doctors' families, can afford that.

But the good thing is, all the national universities were very cheap. Recently, it's not so cheap, but at the time. I had no choice other than to go to the national university. But especially Tokyo University was good because it's a really good school, and I didn't need to move. It's in Tokyo, and I could get there in only forty, fifty minutes. So that's one of the reasons why I chose the University of Tokyo.

MEJIA: They have a good transit system, right?

KURO-O: Yes, right. Yes, a very good public transportation system.

MEJIA: I do want to talk more about your education in a minute, but now I'm just curious, did your sister go to university, too?

KURO-O: Yes, she went to pharmaceutical? What is that in English?

MEJIA: Same word. Same word: pharmaceutical.

KURO-O: Yes, same area. She went to the private pharmaceutical university, and she graduated. Currently she is a housewife, but she had some part-time job with a pharmaceutical company that was over there. So, yes.

MEJIA: Okay. We'll go back to your schooling, then. As you were getting into medical school, any particularly inspiring classes there that helped you pick a direction?

KURO-O: Yes. Actually, I was interested in cardiology. It's because my father had a myocardial infarction when I was in the second year in medical school. At that time, I still had no education about clinical things. He survived. But that was a very good opportunity for me to get into the cardiology area a little bit more. There was a lecture on myocardial infarction, and a professor Yoshio Yazaki taught us about myocardial infarction. Actually, I was very impressed with his talk because there are several ways to diagnose myocardial infarction: electrocardiography, of course; and there are several markers in the blood. Usually at the time people used creatine kinase as a marker for myocardial infarction.

At that time, the professor found a new marker for myocardial infarction, and he tried to apply that marker to the clinical application. I was impressed with his talk, and I just visited his laboratory and spent some time in his laboratory to work on the basic aspects of myocardium. His laboratory is, of course, a clinical laboratory, but he's also very interested in the basic aspects of myocardial contraction and myocardial differentiation and things like that. So I spent some time in his lab and did some basic research in his lab. Fortunately, I got a good publication there when I was still in medical school. That was the, maybe, first involvement in basic research for me, and very fortunately, I could get a good paper published. Yes, I was still interested in clinical stuff, but I was also getting involved with basic research. Actually, I didn't know how basic research was actually going at that time, so it was very, for me, a good opportunity to experience what was really actually going on in the basic research laboratory.

MEJIA: So did you just approach him after a lecture? How did you go from hearing him and being interested, to getting to work in his lab?

KURO-O: Actually, it's a very good system in Tokyo University. During summer vacation of the second year as a student, we actually had extra a one month longer summer vacation than

the other years, from the beginning of June through the end of August. The medical students in the second year are encouraged to visit a laboratory, any laboratory or any clinical department, to participate in some way in the basic research activity or some clinical activity.

Almost all students use that activity and spend the entire summer in a department or laboratory. Actually, it was just before the summer vacation, so I spent that summer vacation in his lab.

There is a coordinating committee so the laboratory can accept such students by applying to the committee, and students who want to do some research can, of course, apply to the committee, and the committee matches them to each other. So there is such a system there. I took advantage of the system, and I went to his lab and did some very basic research on myocardial differentiation.

MEJIA: That was right after you finished your first two years of medical classwork?

KURO-O: Yes. Right. Right. At the time, I didn't know very much about clinical things, but it was really a good experience for me. In his lab I got some idea how basic research was going, and I really liked the basic research laboratory. But I still had two years to go to get my M.D., so I continued medical school and graduated.

Maybe 90 percent of students went to some clinical department for two years. It was a kind of internship or something. And only 10 percent of students went to basic research. It was probably because we didn't have a good opportunity to be exposed to the basic research laboratories, although there were some opportunities in summer vacation in some particular years. But after that, we didn't have direct access to the basic research laboratory. So at that time, 90 percent of students went to the clinical department, and only 10 percent directly went to basic research.

MEJIA: Is there an option, for those final two years, to be in a basic research department?

KURO-O: Yes, there is an option, but almost all students take the examination to get their medical license, and after that, we can choose which way to go.

MEJIA: So you, for those two years, were one of the majority that went into a clinical department?

KURO-O: Yes. Actually, I went to the internal medicine cardiology department, and yes, I was really a clinician in the first, say, five years. I rotated in several hospitals and worked for CCU

[coronary care unit] or looked at lots of patients with acute myocardial infarction or something like that and spent five years. But it's actually a tradition in Japanese medical society to do some basic research and get a Ph.D. At that time I was a cardiologist, but I was also interested in the mechanism of hypertension. So after I got the Ph.D., I had a chance to get some support from the government, and I could spend some time at basic research.

MEJIA: Okay. Can I jump in for a minute?

KURO-O: Yes. Sure, sure.

MEJIA: So you had two years that are like a residency here, it sounds like?

KURO-O: Yes. Right.

MEJIA: Can you describe on the tape at what point you decided to get a Ph.D., and then how that works, since you had already gotten your M.D. It's a different system. You had said that it's not like an M.D./Ph.D. program in the U.S. Can you describe how that worked?

KURO-O: Right. Okay. So after graduation and also during the two years' internship, it was too busy to do basic research, so I just focused on clinical work. After finishing my internship, I continued patient care, but I also studied some basic research, this time smooth muscle cell differentiation. It's a little bit complicated. Okay. So one, two, three, four years of five, six. After graduation I spent two years as an intern, and actually, just before I graduated from medical school, I had a chance to visit the University of Vermont.

Actually, one of the postdocs [postdoctoral fellows] of the laboratory where I spent my summer vacation visited the University of Vermont for four years. Actually, I stayed at his house and had a chance to look at how the research was going on in the United States. It was only two months' experience, again during summer vacation. His name was Dr. [Ryozo] Nagai. He's a key person for me, Ryozo Nagai. Actually, now he's president of Tokyo University Hospital. He's very influential in the Japanese Medical Society right now. When he was a postdoc, he spent some time at the University of Vermont, and I had a chance to be there for two months in summer vacation. He liked me, and I liked him, so our research interest was very similar. He was working on smooth muscle cell differentiation at the time.

But I had to go back to Japan and finish my school program and get a medical license, but he told me that, "When I'm back in Japan, let's work together again," something like that. So he went back to Japan, maybe when I was in the fourth year. After finishing medical school and spending some time in the hospital, he came back to Japan and asked me to come back to

Tokyo University. He was starting his laboratory at Tokyo University, so I joined his lab. That was actually a major change in my career. Of course, in Tokyo University you have to look at patients too, but you can spend more time in research activity, basic research activity.

So we worked together and get some good publications about the smooth muscle cell differentiation. So he was the person who brought me back to the basic research.

MEJIA: You know, I'm going to actually flip this really quick before my next question.

[END OF TAPE 1, SIDE 1]

MEJIA: This is Robin, and we're now on side two, continuing on the first day of the interview.

So you were saying, I guess, it looks like your second year after you finished your internship, so your fourth year of working as a doctor, is that right?

KURO-O: Right.

MEJIA: That brought you back to Tokyo University to do research.

KURO-O: Yes.

MEJIA: So at this point you have your M.D. How are you funded at this point?

KURO-O: Well, during the internship, the university hospital can pay our salary, but after finishing internship, we have to get some money outside of the hospital. Anyway, I worked for some clinics somewhere in Tokyo and get some money just for pay for my bills. But I spent the rest of the time to take care of the patient in the university hospital and spend some time to do basic research. Many M.D.'s just finish internship in Japan, did the same way, so it's a little weird, but it's a tradition in the Japanese medical society.

MEJIA: That makes sense. So then you're back at the university and you're doing research again. When you came back, did you know you were going to pursue the Ph.D., or was that a decision you made there?

KURO-O: Right. All M.D.'s want to get Ph.D., actually. It's sometimes difficult because to get Ph.D., M.D. have to spend significant amount of time to basic research, but it's usually very difficult when they are looking at the patient. But when you stay in the university, we can somehow do that, because a lot of doctors there and they can share their duties and make some time to focus on basic research for a while. So it is essential for M.D.'s to stay in the university if they want to get Ph.D., so I stayed in the university and spent a couple of years for basic research, but I cannot spend 100 percent of my time to research. Maybe, say, 50 percent of my time to research and 50 percent of time to looking at patients. But somehow I got some publications and combined them to a big article.

MEJIA: Like a dissertation?

KURO-O: Yes, dissertation, and applied to the committee and my Ph.D. was approved in 1991. So it took six years, maybe, after I graduated the medical school, to get the Ph.D. At that time I spent maybe more than 50 percent of time for patients' care. It's not so bad for me, actually. Anyway, I got Ph.D. in 1991.

MEJIA: I think you said before we had the tape on that because you had completed an M.D., it basically was just the dissertation at that point.

KURO-O: Right.

MEJIA: You didn't have to go do another series of—

KURO-O: Right.

MEJIA: Okay. Can you talk a little bit about the research interests you had at that point and what you worked on with Dr. Nagai?

KURO-O: Yes. So Dr. Nagai's main research interest was how smooth muscle cells in vessels change during the arteriosclerosis or hypertension. So my research interest was also similar, and I wanted to know the mechanisms, how hypertension develops.

So at that time a new technology, transgenic mouse technology, has become available, so he and I wanted to introduce that transgenic mouse technology to his lab, so I went to another laboratory to learn that transgenic mouse technology. The institute I went was National Institute of Neuroscience in Japan. I tried to make transgenic mice that overexpress an ion channel called

sodium proton exchanger, because at the time the overexpression or activation of that ion channel might be related to blood pressure elevation. So I wanted to make transgenic mice that overexpressed that particular ion channel and wanted to ask whether they develop hypertension. So I spent—I forgot how many I spent, but I spent a couple of years to make transgenic mice and analyze it there. As we expected, they developed hypertension when we load sodium to them.

MEJIA: So you developed mice models yourself at the institute?

KURO-O: Right.

MEJIA: How long were you at the institute? Did you then go back to the lab and work on that?

KURO-O: Well, actually, I was not allowed to spend 100% of my time for the transgenic mouse experiments. I looked at patients in Tokyo University and I went to the National Institute of Neuroscience after I finished my clinical duty there. So it's really tough. So I worked literally fourteen, sixteen hours a day, seven days a week, for several years. It was very tough for me, but it was really good chance for me. At the time, transgenic mouse technology not so popular. It was rather regarded as a high-tech technology. Of course, basic research, I can do that, but nobody thought that clinical researcher can do that, but it was good for me to get the technology and we can get the expected result. But what was good for me was I got some byproduct in that project, and that's brought me here, actually, the science project, so it was really lucky for me.

Okay. So the way you make transgenic mice, you inject gene fragment into mouse egg, and the gene fragment get integrated into mouse genome, and of course we expected that the integrated gene will be expressed and generate more protein and cause some disease or phenotypes in the mouse, but it doesn't usually go like that. Okay. So the gene you injected will integrate into the mouse gene, but sometimes it's not expressed; it's just sitting there and do nothing. So it's a very common phenomenon when you do the transgenic mouse experiments. Actually, I made thirty independent transgenic mouse lines, but only three of them expressed the integrated transgene.

So using those three lines, I found that the overexpression of the gene caused hypertension. But the rest of 27 strains had transgenes integrated into the genome, but the transgene did nothing; just sitting there. But it's theoretically possible that the integration of the transgene could destroy the endogenous mouse gene at the integration site, and if the transgene accidentally integrated into one of the very important genes for the mouse, then the mouse may develop totally unexpected phenotype, not because of expression of the transgene, but because of disruption of the endogenous mouse gene. That phenomenon is called insertional mutation. So I just examined the rest of the 27 transgenic mouse lines, that did not express the transgene, and found that one of the transgenic mice developed disease similar to aging, human aging. It's

just an accident.

So nobody can control where the transgene is integrated, so what we can do is just inject the gene fragment in the mouse egg and let them integrate the transgene. So the transgene will be integrated randomly into the mouse. But it could accidentally hit the endogenous mouse gene and disrupt it. Actually, this kind of phenomenon has been known for years, but it really depends on luck. So if you get some interesting mutant, we can pursue that, but we cannot do that on purpose, actually.

MEJIA: So at the time when you, I guess, were first in the lab, I looked through your publications, it looked like at that point you were still publishing on hypertension.

KURO-O: Yes. Right. Right.

MEJIA: And [unclear] stuff. So you kind of pursued that [unclear] first..

KURO-O: Right.

MEJIA: At that point did you get the mutant mouse?

KURO-O: I noticed that I got mutant maybe about 1992 or ‘93. Just after I got the Ph.D. I went to the National Institute of Neuroscience. One year after I got several lines of transgenic mice, so I noticed there’s some very real mutant there, maybe ‘92 or something like that.

MEJIA: Okay. I definitely obviously want to come back to that, but I’m curious, because you were just describing your fourteen-hour days, when did you meet your wife?

KURO-O: I think it was hard for her. Anyway, but we have to do that because doing both clinical duties and basic research is too greedy, and I never do that again, but I knew that it won’t continue forever, just for a couple of years.

MEJIA: Did you know your wife prior to that period? I know you weren’t married yet, but did you knew each other prior to that period?

KURO-O: I’d leave the house around seven a.m. and be back at twelve midnight, something

like that, but, you know, at the time Japanese people, many Japanese people are workaholics, so it's not very unusual. It's pretty unusual, but not very unusual, actually. My wife is rather conservative type of woman, and she knows that all my father's generation works very hard, very long to recover from the damage of World War II, so many people in my generation looked at the parents working very hard for a long time, so for us it's not very unusual, but still shouldn't be very hard. I don't know the younger generation in Japan can accept that, anyway.

MEJIA: Did you two [his wife, Kumiko Kuro-o, and he] know each other before this really crazy period?

KURO-O: Yes. I got to know her when I was in the last year of medical school. We didn't get married during the internship because I knew it was a really crazy period. I think in the [United] States internship is a crazy period. So we waited to get married after I finished the internship.

Yes, she understood very well what I had to do in that particular period of my career, and her father is also hardworking, a very typical Japanese. I really appreciated that at that point. But we didn't have so very difficult a time to get through that hectic period.

MEJIA: And she was an accountant?

KURO-O: Yes. She had a part-time job at that time, and she could spend some time outside of the house. If she had been a housewife at the time, she might have gone mad. [mutual laughter] She also had work, so we both were pretty busy. So that was okay.

MEJIA: And you two lived in Tokyo still at that point?

KURO-O: Yes. Yes. We never were apart.

MEJIA: At that point, then, you go back to basic research and prepare a dissertation.

KURO-O: Yes.

MEJIA: Once you have a Ph.D., what options does that open up? You described supporting yourself and then being able to do the research. What options does getting a Ph.D. open up?

KURO-O: Yes. After getting the Ph.D., there are actually still essentially two options. One was, of course, continue looking at patients and doing basic research at the same time, basically continuing the lifestyle. But of course, once you get a Ph.D., you have to train the younger researchers. There is some change in the role, but essentially you can go along in the same way. That's one choice.

Of course, you can choose to do a completely different thing like I did: intern, go to basic research. But it's not very common, I think, because usually, once you drop out of the academic career in Japan, it's very difficult to get back again.

I think the United States is very unique because actually, in Japan, age is a very influential factor to determine one's position. So usually, let's say, the Ph.D. in his forties cannot start as an instructor. Usually they expect to be an associate professor. There is a very strict hierarchy in the Japanese medical community, and postdocs [postdoctoral fellows] may be, at most, in their thirties, assistant professors and associate professors in their forties, for a professor, fifties, and usually it's not very common to work fifty years old as a postdoc under the supervision of a thirty-year-old assistant professor. That couldn't happen in Japan.

Then, naturally, as you age, you have to get a certain position. So that's how it's very difficult to change a career in the middle of the career. Let's say, if you spend years as a clinical doctor and as you get to forties, then it's real difficult to change one's career to a basic research area, because in your forties, people expect that they should be an associate professor who already has his own lab. So he cannot start from scratch, like a postdoc or a student.

Anyway, so when I got the Ph.D., I was thirty-one. So I had to decide which way to go. If you stay in the clinical department, yes, that's one way. But if you want to go to basic research, I had to decide right now, immediately, because if I spent several years in the clinical department, I would be thirty-five, thirty-eight, and at that age maybe I could not transfer to the basic research area because I had to be assistant or associate professor at that time.

I was thirty-one. This was probably the last chance to change my career in Japan, and I really wanted to work on that mutant I found. The mutant looks aged. It's theoretically possible that the integration of the transgene could destroy the endogenous mouse gene at the integration site. That phenomenon is called insertional mutation. Because of the transgene mutation, it could accidentally hit the endogenous mouse gene. So I really wanted to understand what gene was disrupted and what gene disruption caused the similar to aging in humans. I could identify the gene disrupted in that particular mutant. That gene [*Klotho]* could be an aging suppressor gene, because if you disrupt it, animals get old very quickly. If you could identify that gene, the function of that gene could be suppressing aging. That could be a huge discovery. I decided to pursue that area. I decided to spend my time and focus on the identification of the gene disrupted by the transgene integration. So I moved to the National Institute of Neuroscience, where I developed the transgenic mouse and spent time identifying the gene.

MEJIA: Is this like a postdoc arrangement?

KURO-O: Yes, as a postdoctoral fellow.

MEJIA: So you were funded?

KURO-O: Yes. I was funded from NIH [National Institutes of Health] in Japan [National Institute of Neuroscience], so financially it was fine. I was paid and got a grant, so I was able to somehow survive, for several years, at least.

MEJIA: And you were able to just do research at this point?

KURO-O: Yes. At that time I spent 100 percent of my time doing basic research. Actually, identifying the mutated gene in the mouse genome is very laborious work, so I knew that I couldn't do that doing clinical duties together. So I gave up the clinical duties, and I left Tokyo University and moved to the research institute.

MEJIA: So you were able to answer your original questions using the mice, that had worked?

KURO-O: Right. Right.

MEJIA: And is it fair to say you were most interested at that point in your strange mutant, or were you still working with those other ones?

KURO-O: I was still working on that genome, okay? So it took maybe four or five years to identify the gene, and I named the gene *Klotho* after the Greek goddess who spins the thread of life. Anyway, I could identify the *Klotho* gene, and I got a good publication about the *Klotho.*

MEJIA: You got a very good publication.

KURO-O: Well, it was very lucky for me, and that paper actually brought me here [University of Texas Southwestern Medical Center].

MEJIA: Which I want to get to, definitely. Could you just maybe talk a little bit more about

what it was like at the institute [National Institute of Neuroscience] in your postdoc? Were you working under somebody in the lab, somebody here? How independent were you?

KURO-O: My position was postdoctoral fellow, but the reason why I first visited that institute was to learn transgenic mouse technology. There was no particular scientific supervisor, actually. I just wanted to learn how to make a transgenic mouse. And of course I brought my project, both of my projects, to make transgenic mice that overexpress sodium-proton exchange. So, scientifically I was totally independent, but financially I had to borrow some money to get the reagents necessary to do the research or borrow some equipment or space to carry out the research.

I belonged to the molecular genetics department there, but I received no salary from them. I provided my entire grant to them to get some reagents from the grant, and of course, it was not enough to support everything I spent, so I borrowed some reagents, many from them. So financially, they contributed very much to my work. Of course, the results we shared, the office shared with them. I was very happy to work with them.

MEJIA: Can you describe just a little bit what the process is? You know you've disrupted a gene somewhere, and you're interested in it, but what do you do?

KURO-O: What I actually did to isolate the gene?

MEJIA: Yes.

KURO-O: Okay. In the mouse genome, the transgene was integrated somewhere. What I had to do was identify the gene just around the integration site. So the first thing I did was to isolate the genes close to the integration site. It's easy to say but difficult to do. Anyway, I can somehow identify the genome sequence adjacent to the integration site.

And the next step is to compare this region to the original mouse genome and find out what is different between them. Sometimes the insertinal mutation occurs frequently than we thought, because some people say that 5 percent or 10 percent of all transgenic mice show some kind of phenotypes related to the insertional mutation. It's not a very rare phenomenon, but people never pursued or people never tried to identify the gene disrupted by the transgenic division because integration of the transgene usually results in severe destruction of chromosomal architecture.

Usually it's very difficult to identify what gene was lost or what mutation was induced because of the huge destruction of the overall chromosomal architecture. But I was again lucky because transgene integration generated a very tiny deletion, simple deletion, at the integration

site, and in other parts, it was almost intact. So it was not easy, but relatively easy to identify what was disrupted in the mutants.

Anyway, we found out that the transgene integration produced a small deletion in the original mouse chromosome. It's like this. There is a mouse genome and there is the mutation of the transgene, and there is a small deletion here. Something is lost here. So maybe there might be a gene here, and the rest of this gene could cause the aging-like phenotype. So I just tried to identify it. There might be some gene around here, and I can find one gene very close to this deletion site, and actually, it later turned out to be a *Klotho* gene.

But the difficult thing is what do we know about. The transgene was integrated here and this part is lost. That's fine. You find the gene here. That's fine. But there might be several genes around here, and deletion might somehow influence the expression of other genes, and these genes could be the true cause of the aging-like phenotype. We cannot exclude that possibility, but we cannot search everything around here. But anyway, I could identify one gene, and this gene expression was very much suppressed in the mutant. So that gene was a very strong candidate for the responsible gene of the aging-like phenotype.

Okay. If that gene is indeed responsible for all the aging-like phenotypes in the mutant, we may be able to rescue that aging-like phenotypes when you bring the gene back to that mutant. So we did it, and indeed, the aging-like phenotypes are completely rescued. So we were able to conclude that this gene I isolated was indeed responsible.

MEJIA: So you reintroduced the gene through a vector?

KURO-O: Right. Yes. It's actually a long way, so to identify the gene, it took maybe two or three years, and to prove that this gene is indeed responsible for the aging-like phenotype, we had to make, again, transgenic mice that overexpress this gene and cross them back to their mutant background and see whether the transgene can rescue the phenotype. It was a long study, but it was successful, so I was pretty happy to get this data.

MEJIA: When did you name the gene?

KURO-O: Well, when I isolated this gene. It was maybe '96 or '95, and I spent another year to prove that this is indeed the age suppression gene, so my publication was '97 or maybe '95 or something.

MEJIA: The name is elegant. I'm just curious, did it just come to you? How did that happen?

KURO-O: Actually, my ex-boss, the chief of the molecular genetics department in the National Institute of Neuroscience, his name is Dr. [Yoichi] Nabeshima, and he was not directly involved in the scientific part of this project, but he was very curious about what I was doing. He read some books on Greek myths, and he picked out that name. Klotho in Japanese sounds a little bit like my name.

MEJIA: Oh, I hadn't thought of that.

KURO-O: Especially we cannot distinguish the sounds of *L* and *R.* Japanese has no *TH,* so in Japanese, Klotho and Kuro-o sound pretty similar. That's why he picked out this name, maybe.

MEJIA: Up to that point you had some pretty good success with publications, but this was obviously a breakthrough. At what point did you think this might be a *Nature* paper?

KURO-O: Actually, at that time, maybe there was no example for single-gene mutation causing aging -like phenotype. That was one thing, I think. And another thing is, actually, identification of the gene caused by insertional mutation was pretty tough at that time. So technically it was challenging. That may be another reason.

Maybe it's because a single gene may regulate aging. Recently it's not a very new concept, but at that time I think it was pretty new. Of course, some people argued against me because these mice developed many aging-like symptoms, but they might simply be sick and develop many diseases, and it may have nothing to do with aging. That's a very reasonable criticism, I think. Actually, I spent the next four years arguing against that.

MEJIA: How did the writing process go for writing all that up? Was that done with other people, or was that you?

KURO-O: Yes. I wrote it down by myself, and yes, it's not very difficult. It was a really straightforward study, just simply spend a lot of time, I spent a lot of time, but it's very straightforward. I made the mutant, the mutant showed this kind of phenotype, I identified the gene, and I rescued it with the aging -like phenotype. That's pretty straightforward and simple, logical sequence, so it was not difficult, and I also had it checked by the native speaker. But essentially I wrote it by myself.

Actually, after submission, *Nature* editorial office extensively revised my writing. Actually, the original article I submitted was a series of three papers for phenotyping. The first article just described the aging phenotype, the second paper identified of the gene, and the third paper, rescued the mutant phenotype. But *Nature* wanted us to combine everything into one

single big paper. So when I submitted it the first time, there were a total of twenty-seven figures and tables, but *Nature* asked us to compress it to six figures and tables. Somehow we did that, and *Nature* liked the new style, so it took not a very long time to be accepted.

I was actually very worried about whether they could accept the concept that this was a model of aging. That was a point, I thought. But fortunately, the reviewers agreed with my opinion. In technical part, I think there was no room for discussion. It was very straightforward. But the interpretation of the results regarding these mice as an aging model could be a little bit challenging. Fortunately, the editors and reviewers accepted that interpretation.

MEJIA: Forgive me if I'm wrong, because I'm not very technical, but in reading up on your work before this, one point struck me as interesting because it looks like your mutant produced symptoms more similar to human aging than to mouse aging.

KURO-O: Right. Exactly right. So even if you keep the mice for a long time, they never develop phenotypes like *Klotho* mutant. Phenotypes that develop Klotho mutant mice are very similar to human aging-like phenotypes, like arteriosclerosis, osteoporosis, emphysema, skin atrophy, and many other features of human aging. So, right, it's not the acceleration of natural aging process of the mouse, but it's some mechanism that might be related to human aging or human-aging-related disorders. That's another very important point, I think. So it shouldn't be regarded as acceleration of mouse aging; it should be regarded as a model of human aging.

MEJIA: Then this fairly big paper comes out in *Nature.* Can you describe what that was like for you in your career?

KURO-O: Oh yes. It totally changed my life. So I didn't expect that publication in *Nature* could cause such a big impact in every aspect of my career. Yes. I just wanted to publish these findings, maybe *Nature* would accept it. That's the only reason why I submitted my paper to *Nature.* Any paper would be fine. *Science* would be also a great journal, or even they might reject us, but I was pretty happy if I could put my data in any journal. That was what I thought at the time. Actually, I was surprised that the institute planned the press conference. I never expected that, but they indeed did a press conference, and it was good advertisement for the institute and also a good advertisement for the grant program that supported me at that time, but it was a really interesting experience for me.

From a scientific point of view, this paper was a really interesting paper, but identification of the gene was not, of course, good enough to understand the aging mechanism. I thought it would be very important after I found the gene. To identify the gene is one thing, but to identify its function is another thing.

What I had to do next was to identify the gene function, or function of this gene, or

function of this gene product, Klotho protein, but I had no clue how to address that question at the time. I was pretty happy when I got published, my paper, in *Nature*, but at the same time, I felt a little bit of anxiety because I had no clue to the next step at that time. But the impact was really high. Many people wanted to study the *Klotho* gene, but there was no clue how to analyze Klotho function at that time. So, yes, that's what I felt at that time.

MEJIA: And you had developed the mice, so I guess you would have been the source for everybody's inquiries? People would have come to you if they had questions about how to try and study this?

KURO-O: Yes. Right. Other people wanted to use these mice for their own research, that's right. Many people working on, say, aging. So one might want to see what changes would be observed in the central nervous systems of people who are working on Alzheimer's, of course, are interested in this aging model.

Of course, every organ has some phenotype, so, yes, that's one we actually saw, the phenotype in each disease, observing Klotho more precisely, more deeply, and understanding why this happens or to find out the mechanism.

That was one way to go, actually, but I didn't want to go that way, because I would rather leave those projects to them. But rather, I'd like to focus on what is the fundamental function of the Klotho protein. That was really the challenging part, that maybe I should work on that.

MEJIA: We're going to break and continue tomorrow.

[END OF TAPE 1, SIDE 2]

[END OF INTERVIEW]

INTERVIEWEE: **Makoto Kuro-o**

INTERVIEWER: **Robin Mejia**

LOCATION: **University of Texas Southwestern Medical Center Dallas, Texas**

DATE: **7 March 2006**

MEJIA: This is Robin Mejia here with Makoto Kuro-o on March 7th, 2006, in his office at U.T. [University of Texas] Southwestern [Medical Center] for the second day of the Pew [Scholars Program in the Biomedical Sciences] oral history interview.

So when we left off yesterday, you had just published the results of your five-year intense work in Japan, and that was about to lead into many big things. But when I was reviewing the tapes, I just had a couple of questions that I wanted to start out with before we jump back in there, which is, at point during your schooling did you learn English?

KURO-O: All Japanese students learn English at the junior high school, so age twelve, thirteen. In high school, students usually take English courses, and some students take French classes or German class, but maybe 99 percent of the students take English classes in high school, too. So a total of six years, three years junior-high-school education, three years high-school education, so a total of six years of English classes.

But the problem is, teachers are all Japanese. So, yes, I think probably Japanese are probably very good at English education in Japan, very good in how to read English, how to write English, but speaking and hearing is another thing. So that's why most Japanese are not very good at speaking and hearing English, but probably very good at grammar and spelling and writing or reading, maybe.

MEJIA: That makes a lot of sense. Those are two different things, and written English isn't always phonetic, either.

Six years was enough so that when you were at a point where you wanted to publish and needed to write, you'd be able to more or less do that fairly independently then?

KURO-O: Yes. Of course, nobody uses English there, so if you learn English in high school, usually you lose everything if you don't keep reading or writing English. But in medical school, we sometimes use English textbooks, and of course, after graduation we need to read English papers, so we have a chance to continue to be exposed to English, reading especially. So that

helped me a lot to write up my own article.

But, you know, all English I read in medical journals, so I didn't know anything about conversation or how to, say, order in McDonald's [restaurant], check into a hotel, or something like that. We didn't know nothing about that, but knew how to write up scientific papers. I think many Japanese scientists and medical doctors can somehow write or read English journals, but of course, they are not very fluent.

MEJIA: Right. Well, I imagine staff at major journals are probably used to working with people on their English when they need to.

KURO-O: Yes. Actually, *Nature* editorial office helped me a lot, maybe almost each sentence, sentence by sentence, and, yes, it's actually very different from my original writing. But that made the article much shorter and more comprehensible, understandable, readable, easy to read for many people. So that's good.

MEJIA: And this brought up another question I was thinking about. During your medical training, initially you said you were very focused on the clinical side, and that was your intent, was to be a clinician and doctor. How much were you tracking journals and research during that point? Did you have time for that?

KURO-O: Yes. After graduating medical school, during the internship, I didn't have any time to track basic research journals, but when I came back to Tokyo University, I started some basic research, so naturally I needed to read some related topics. At that time there was no PubMed or there was no good database for journals, so we just read the special journals. At that time I read, of course, *Nature, Science,* something like that, but I was interested in cardiology, so I read some journals like *Circulation Research* and *Circulation* or some specific journals. I had some time to read such papers, but maybe it was a little bit biased. We didn't have any good database such as PubMed at that time, but yes, something like that.

MEJIA: Two more here before we come back up to time. You talked about visiting Dr. [Ryozo] Nagai in Vermont, but how did you meet him originally? Did you know him from your time here?

KURO-O: Yes. I told you that I spent some time in a research lab when I was a medical student, summer vacation for a couple of months, and he was in Japan at that time. He left Japan just maybe a couple of months later, but at the time he was still there, I had a chance to meet him. But at that time, of course, I just met him. But he went to the University of Vermont, and actually, the boss Yoshio Yazaki of the laboratory gave me a chance to go to the University of

Vermont because Dr. Nagai was there, and I spent two months there at the University of Vermont. So that was the first time for me to closely interact with him. So this boss actually had me come to meet Dr. Nagai.

MEJIA: He may have talked with his boss and said, "I know this doctor from Japan who's really smart," and, "Can we bring him over for a couple of months?"

KURO-O: Probably boss said, "I have a student that wants to visit the United States," something like that. So, yes.

MEJIA: And so that's when you two got to know each other, which led to him pulling you back towards research here?

KURO-O: Yes. Right.

MEJIA: And then moving up closer to where we were yesterday, I was struck by this when I was looking at your papers beforehand and again when I was listening to the tapes. It does sound like you really took off on a pretty novel path after you got your Ph.D., when you decided to figure out which gene had been knocked out in your mutant. Was that a big decision? Was that just self-evident? What convinced you that you could go do this thing that nobody else was doing?

KURO-O: Right. To tell the truth, at that time I didn't feel that I made a big decision, because I mixed mice and found a very interesting phenotype, so naturally I just wanted to know what was going on in that mouse. To do that, I had to give up clinical duties. So that was a very natural way to go. But after several years later, when I thought about my career, I realized that that was really a big decision. At the time I didn't realize that that would be a big decision, because I just wanted to know what was going on in that particular mouse. But that required me to change my career. So that's why I did that. That's it.

MEJIA: You described fairly eloquently yesterday what you did to find that gene and determine that it was responsible for what you saw. You also mentioned you were working pretty independently at the National Institute [of Neuroscience of Japan] at that point. How did you go about finding the tools and techniques and figuring out what to do next? Was that from just talking to other researchers there or from literature? How did you determine your course of action?

KURO-O: I explained in the design or something like that?

MEJIA: Yes. How did you design the experiment that sounds so clear and obvious when you explain it after the fact?

KURO-O: Right. I had a background in molecular biology, so cloning of the gene was technically not very challenging for me, but of course the sequence I needed to look at was fairly big, so I thought it would be very laborious work, but basically all the techniques necessary for the identification of the gene, I just used the conventional molecular biological technique that I learned from Dr. Nagai. So technically it was not very challenging, but the amount of the sequence we needed to analyze was pretty big, so that took me a lot of time and paper. So, yes, you can get some genomic fragments. It's not so very difficult to get the genomic fragments surrounding the insertion site, but we needed to know its portion includes the actual protein, the exon. So identification of the exon in this region took almost two years, maybe, for me to identify the potential sequence. I used the technique learned from Dr. Nagai, and the methods I just got from literature, old literature.

The good thing is that in the Department of Molecular Biology had several faculty members, and one of them, two of them, worked on flies, fly genetics. They were routinely doing that kind of work. In fly genetics, they pick up some interesting mutant and identified which gene was disrupted in that fly. So it was completely the same thing in fly. But in mice, the genome size is much bigger, so the analysis is a little bit more complicated. But theoretically, the principle of the experiment was almost the same. So I sometimes got some advice from such fly guys. That was very helpful. That was very lucky for me, too. The fly geneticists [Drs. Fumio Matsuzaki, and Chihiro llama] were next door from me, so that was very lucky.

MEJIA: Okay. That makes sense. So then moving forward to where we were yesterday, which is you'd gotten the results and the paper came out, and just before we wrapped up, you were just starting to explain the reaction of the scientific community, what happened next in your career.

KURO-O: Right. Well, at that time there was no mammalian model for aging, so probably the *Klotho* mutant mouse was the first example of human aging in general. Actually, there were a couple of aging model mice at the time. It's called the senescence-accelerated mouse, SAM, SAM mouse, but they are a natural mutant, and the inheritance of the aging phenotypes are very, very complicated, probably because, they are not caused by single gene mutations. So nobody knows what genes are mutated in that particular mouse, or more importantly, there are several substrains in SAM mice, and each substrain expressed some other aging-like phenotype. So any single substrain expressed multiple aging-like phenotypes. The unknown characteristic feature of aging is that a single individual gets multiple diseases. That's a very characteristic feature in aging.

But these mutant mice did not have such multiple phenotypes in the same individual. In that sense, the Klotho mutant mouse was the first example of aging. But the impact was, I think, really big, but at the same time, nobody knows what the gene is doing, I mean what the Klotho protein is doing. Nobody knews at that time. So everybody thought that was a very interesting finding, but there was no clue to the next step at that time.

So after publishing the *Klotho* gene paper in *Nature*, there was no major publication probably for more than several years. I provided Klotho mutant mice to many researchers, and they were interested in particular phenotypes. I gave it to an ophthalmologist and he looked at whether they had cataracts or not. I gave the mouse to, say, a cardiologist, and he look at how the arteriosclerosis was going on, something like that. But it was a kind of descriptive study and not getting too deeply into the mechanism of the disease or Klotho protein function. So, yes, it was good I could publish the paper, but the next step was pretty much invisible at that time.

MEJIA: Scientifically the next step was invisible, but maybe could you talk a little bit about what happened next in your career? Were you invited to talk on this to people? Did you become more of a name suddenly?

KURO-O: I got invitations from many institutes at that time. At that time I was a postdoc [postdoctoral fellow], so naturally, I wanted to get to be assistant professor or want to have my own lab, independent laboratory. But, I didn't know, in Japan, everybody has got to get totally independent before age, say, forty. Actually, I said I belonged to the molecular biology department in the National Institute of Neuroscience, and the chairman of that department was Dr. Nabeshima, Yoichi Nabeshima. He's a Ph.D. and he is a basic scientist, and he worked on muscle development for a long time, but he was also very interested in Klotho mouse. He actually got very famous because of the Klotho mutant mouse. After publishing the *Nature* paper, he planned to move to Osaka University, and he offered me a position under his control. I couldn't be independent if I accepted his offer. So I wanted to be independent and I wanted to have my own laboratory, but it might be too early for me in Japanese society. So I almost accepted his offer, but when I came to U.T. Southwest and I met Dr. [Errol Clive] Friedberg, the chairman of this department, he offered me a very good independent position and laboratory space and start-up funds.

But I never thought about coming to the United States, and I was looking for a position in Japan, so I never thought about coming here, coming to the States. So I couldn't decide immediately. I need to talk with my wife [Kumiko Kuro-o], of course. But eventually I decided to come here. The main reason was because I would be able to have my own independent laboratory, and, second, as long as I continued Klotho research in Japan, Dr. Nabeshima would be very influential, and all the work I would do in Japan might be not very fairly evaluated. You know what I mean?

In Japan there is a kind of hierarchy, so everybody knows that. The boss never does the

actual experiments, but the assistant professor or postdoc people, whatever, are doing the actual research. But all the publications would be usually corresponding also to that big boss. So in Japan everybody knows the system, everybody knows, okay, the corresponding author is the famous guy, but probably this work was done by the last one or something like that. So in Japan, everybody knows. But if you publish it, if the scientists in Europe or the United States read the paper, 100 percent of the people believe that this work was done by the big boss. So there's a discrepancy between the publication style, and who the actual work was done by.

If I stayed in Japan and worked with Dr. Nabeshima and published many papers, if I did that, still people would believe that this work was done by Dr. Nabeshima's laboratory; that was not actually very fair for me. So I really wanted to get independent from him.

Of course, if I could find a good position in Japan, that would be one choice, but maybe moving to the United States would make the things more clear. If I could publish a good paper from my own laboratory in the United States, the things are very clear that this work was done by myself and not by Dr. Nabeshima, something like that. That is one thing.

But of course, another reason is I really wanted to see what was going on in the United States in the basic science area. I never had a chance to visit United States. Of course, I did visit the University of Vermont, but it was just when I was in medical school, and it was just two months. It was not enough to see everything. So I really wanted to look at how basic research was going on in the United States. So, yes, those are the two major reasons why I moved to the U.T. Southwestern.

MEJIA: That makes sense. It sounded like you said you weren't actively looking in the U.S. [United States], even though you had ended up with the offer. How did you end up coming here for a seminar? I think you said you went to another conference first?

KURO-O: Yes. In 1997, I remember it was November. There was American Heart Association meeting, and I applied for Young Investigator's Award for the meeting. It was in Florida, and I went there. But at that time the *Nature* paper was not published yet. The paper was published November 7th. But Dr. Yanagisawa was in this campus, knew that I was working on Klotho mice, and isolated the *Klotho* gene, and that the paper was accepted to *Nature*, so he invited me to speak here.

I just went to the joint aging meeting and just stopped by Dallas [Texas] and gave a seminar and Dr. [Errol Clive] Friedberg was talking about offering me a position. But I couldn't decide, so I went back to Japan and thought about four months or so whether I should accept this or whether I should still search for a position in Japan. Actually, in Japan there were several professor positions available for me, but I knew that several candidates had applied for a single position, so there was no guarantee that I could get the position. In Japan, the professor is selected by voting by the other professors, so there is a little bit of a political thing. I don't know whether I could be a professor in Japan. But Dr. Friedberg's offer was just yes or no. If I

accepted, I could have the laboratory.

MEJIA: So he made the offer during your trip?

KURO-O: Right. I was really surprised. I didn't know him at all. I first met him and I just talked with him and showed my data, and just talked with him ten minutes or so, but he offered me the position at the spot. I was really surprised. I don't know how he did that. Maybe my work was a little bit related to his research, because he is working on DNA repair systems, and that is actually directly related to aging. So maybe that's why he was very interested in my research.

Fortunately, at that time he got an endowed scholar program offer [Southwestern Medical Foundation Scholar in Biomedical Research]; the Department of Pathology can take one junior faculty using that particular endowment. So he was actively looking for a junior faculty at that time. But that was really good timing for me. That fit his research interest and his project, and, yes, everything fit fine.

MEJIA: That was probably the first year of that program, right?

KURO-O: Right. I didn't know at all about the program, but Dr. Friedberg said to me that I would offer $600,000 for start-up funds. So it's a lot of money. In addition, of course, I could get independent laboratory space. Yes, it's a really good offer, but it's in the United States, so it was a big move for me. I was thirty-eight at that time. Brain is getting more stiff. It's getting harder and harder to learn new things and especially new languages. So that was a really big factor for me when I decided to come here.

I didn't know how the students are educated, what education was going on in the States. I had no idea about how the granting system works in the United States. It's totally different. But anyway, his offer was really good, so I could not resist.

MEJIA: And how did your wife [Kumiko Kuro-o] react?

KURO-O: Well, at first she really didn't like to move to the United States. She is thirty-seven, one year younger than me, but still thirty-seven. Unfortunately, she took French when she was in high school. One percent, very rare case. She learned French literature in the university, so she had no English at that time, so naturally she didn't want to be here. But anyway, to improve my career, there was no choice but to be here, so I persuaded her and she eventually said okay.

Of course, she had a very difficult time for the first several months, and she had no

English and no friends. Actually, she never drove in Japan, so here in Dallas without a car, she cannot go anywhere. But there were several Japanese families in U.T. Southwestern, and actually there were some Japanese postdocs in Dr. Yanagisawa's lab and Drs. Brown and Goldstein's lab that helped us a lot. Actually, we knew their family when I was in Japan because we worked for the same department in the University of Tokyo, and actually, my family and his family lived on the same condominium in Japan for several months, so we knew each other very well. So their family helped us a lot. His name was Dr. Shimano, and his wife helped my wife a lot, taking her everywhere, where to buy the food or where to buy the housewares, Target and so on. But that really helped me, actually. So she was gradually getting used to the American style of life.

She also went to community college to learn English as a second language, ESL [English as a second language]. She took ESL classes in the local community college, and yes, she did pretty well, and she graduated two years ago. It was really good for her, and she got some friends there, and she, of course, learned English. Now maybe she is enjoying the life much better than me, maybe. Yes. Women are very strong.

MEJIA: That does sound like a fairly big move.

KURO-O: Yes, indeed.

MEJIA: What did your parents [Masahiko Kuro-o, Nobuko Kuro-o] think?

KURO-O: Well, actually, many M.D.'s in Tokyo University spend several years, maybe three, four years in the laboratory of the United States to learn the experiments, to look at what is going on in the United States. So my parents thought that I was just spending several years, a couple of years, and we'll be back soon. So that's okay, something like that. "It's a good experience for you." But it's already eight years, almost eight years, since I left Japan. So, yes, my parents, they probably think that as long as I am successful here, they will be okay, because eventually children need to get independent from their parents. But they are getting very old, and I'm a little bit worried about it. Actually, my father [Masahiko Kuro-o] had a stroke two years ago, and his left side is a little bit paralyzed, but my sister [Atsuko Rakuman] lives very close to the parents so she can take care of them. That's good for me. Every time I have a chance to get back to Japan to give a seminar, I always stop by my parents' and see how they are doing. But maybe this year I will have a chance to get back to Japan three times. That's good for me. So, yes, that's my only concern in Japan, but otherwise, we think they will be fine as long as I'm happy here.

MEJIA: Okay. I'm going to flip this over here.

[END OF TAPE 2, SIDE 1]

MEJIA: Okay. We're on side two of the second tape.

So could you maybe now talk a little bit about the experience of coming and setting up the lab? So you have this, from what I've read about the [Southwestern Medical Foundation Scholar in Biomedical Research] Endowed Scholars Program, probably a pretty great offer.

KURO-O: Yes, yes. It was a very good offer, and it provided me $300,000 for the first year and $100,000 for the second, third, and fourth years. So in total it's $600,000.

MEJIA: Is that in addition to a salary?

KURO-O: Right. Salary was taken care of by the department. It's a direct cost, so I can use it for research. Well, but I didn't know at all about the system, so actually I hired too many people at first. I hired three postdocs [postdoctoral fellows]from Japan [Hiroaki Masuda, Tasuo Suga, Hirotaka Chikuda], and at that time I had no idea about how much money they spent, including their salary. So three postdocs are a little bit too much for my budget. And I also had a big mouse colony, and that costs a lot to maintain the mouse colony. So actually, the budget was not enough for me. But fortunately, I could get several small grants. Of course, Pew [Scholars Program in the Biomedical Sciences] provided me from 1999; it was the next year after I arrived here. At that time the budget was really tight, so I was really very happy I could get the support from Pew [Scholars Program in the Biomedical Sciences]. That money really helped my laboratory. The budget itself is not very big, but still, for my small laboratory, that was really a big amount of money.

And I got some very pilot program type of small grants from NIH [National Institutes of Health], yes, something like that. President's Research Council Award [President's Research Council Distinguished Young Researcher Award at U.T. Southwestern]. It was actually good money for me, but it was only a one-time fund, grant. So I really needed to get an NIH [National Institutes of Health] RO1, but it was really tough to get the brand-new RO1.

But anyway, when I started the laboratory, the budget was very tight, and I got postdocs from Japan. So actually, nobody knew about the system, right? So I didn't know very well about the system in the States, and the postdocs from Japan, of course, didn't know anything about what was going on in the States. But I got one technician [Rosa Shamlau], and of course, she is from the States, and she helped me a lot to set up the laboratory. Of course, the department supported me a lot. I worked with a secretary [Lisa Jones] who was very experienced. I just asked her, "I want this and this and this and this," and then she placed an order and arranged the shipment and everything. So anyway, maybe it took a couple of months to get everything, all the

equipment I need in the laboratory, and then started the research somehow.

But at the time I had no clue to the Klotho protein function, so I built some hypotheses about the Klotho protein function and we tested that, but every time we tested it, the hypothesis was not working. So there was no data that I could publish. For maybe the first four or five years, I had no good data that could be published in a good journal.

But I continued some collaboration with my friends in Japan, researchers in Japan, and we could somehow get some publications, but I couldn't get a good publication about the data generated in my own laboratory. That was a really tough period for three, four years. And every time I submitted a grant to NIH, it was rejected or got a low score. So that was a very tough year, anyway.

MEJIA: How did you learn to navigate the grant system? Did you work with anyone here to learn how to write NIH grants?

KURO-O: Yes. Actually, the chairman, Dr. Friedberg, helped me a lot. Of course, he got a lot of grants. He just showed me how to write up the grants, an actual example. He gave an actual example how the grant should be written up. And after I thought up the grant, he checked my English and the organization of the writing. So he really helped me a lot. Yes, anyway, I just borrowed a grant that was approved, from several colleagues, and just looked at it and thought maybe I should write it up in this way or something like that.

The good thing in the States is they let me know what was wrong and what was a weakness of this proposal, what was the strength of this proposal. Actually, in Japan there is no such feedback. I just applied for the grant, and I just heared yes or no; there was no feedback about what was wrong. So I thought the grant was something like that. I thought it was different from publishing a paper in the journal and there was no feedback at all. But I could get some feedback from NIH so I gradually understood what was wrong in my proposal and what they required.

I could submit, of course, a revised grant, and the score got up a little bit, but still didn't reach to the funding score. Lots of failures taught me how to write up the grant that could be approved by NIH. So maybe I tried five times and failed five times, and the sixth time the grant was approved. It was 2002, and that was a really critical time for me, because the Endowed Scholars Program ended on that day, on that year. So if I couldn't get any funding by 2002, I had to close my laboratory. There was no overlap at all. I was cleared. But anyway, somehow the project continued that critical period.

MEJIA: So it sounds like the Endowed Scholars Program was actually key for you in doing what it says it would do, which is give people the time to pursue something novel and different.

KURO-O: Right. Indeed. Yes, it's a totally new gene and totally new protein. Of course, nobody knows what this protein is doing. Yes, a good example is APP, Alzheimer amyloid precursor protein. Actually, that protein encodes a membrane protein. The APP protein is actually accumulated in the Alzheimer patient's brain. Everybody knew that a long time ago. APP was isolated a long time ago, but nobody knows what this APP is doing. Literally thousands of researchers in the neuroscience field want to know what this APP is doing, but still its true function is not very clear. So the same thing could happen to Klotho. It's also a membrane protein, and the only data available is when you disrupt the Klotho gene, mice look like aging. That's all we know. So, yes, thousands of researchers worked on APP, but still its function is not known.

In the case of Klotho, I'm the only person working with the Klotho protein, and it's very likely that I couldn't understand anything about the Klotho protein. That was actually the big fear for me, anxiety for me, or concern for me during the first four years. It might be too tough for me to pursue. Indeed, we didn't get any excellent data in the first four years. And budget time was very close, getting very close. So, yes, that was very tough for me.

MEJIA: This is your first principal investigator [position], assistant professor, as you said, you were running your own lab. How did you work with your postdocs? How were research ideas generated? Were they bringing in their own ideas? Were you still directing the ship? Were they bringing stuff in?

KURO-O: The postdocs I hired from Japan were all cardiologists, actually, so they just wanted to spend a couple of years. Of course, if they could do good work and publish a good paper, that would be very nice, but one of the major purposes for them was to learn the American system of science. In that sense, they are not 100 percent committed to the science. That's known. Actually, for them it was a very special period of time. They were very busy once they got back to Japan, taking care of patients and doing many critical duties, but they were allowed to spend two years. Usually two years is the maximum for them. So two years to look at the United States and to be exposed to basic research, so they had a little experience with basic research.

So for such people, I think two years is too short to contribute to science in depth, because for the first several months they needed to spend more time setting up their life in the States and taking care of their families in the different environment. So they spent several months for that, and they spent several months learning how to use the pipette, how to use the machines. After that, they could start research. But they had to get back to Japan two years later. So the actual period they could really focus on science was probably only one year or so. That was too short for them to generate productive data or to propose a good hypothesis. Actually, that was probably one of the causes that we couldn't get good data for the first several years. They are really clever and they have a big ability. If they could spend another year, three years or four years, but two years was too short for them.

Japanese medical doctors spend two years usually in a big lab, established lab. So in those laboratories there are lots of projects going on and there are already established methods or assays, and they just work on one part of the team. Two years might be okay if they work for the very big established laboratory. But in my laboratory, it was not very established. I didn't know what way to go, still searching for which way to go. So in such a small laboratory they might not be very happy, I think, because they couldn't get a good publication during the stay. So it was really a frustrating period for both of us because they couldn't get good data, I couldn't provide a good project. But I couldn't do anything other than that, so I had no choice. Anyway, I had to struggle, pursue any possibility or any hypothesis that might be promising. But as you know, science is not always easy.

So, yes, maybe it was in the third year, maybe, 2001 or 2002, we noticed that the Klotho protein might be involved in the insulin pathway. That was the breakthrough for me. At that time, all the postdocs went back to Japan, and I was totally alone. Actually, I hired one postdoc, but for a few months I was totally alone. Nobody was in that laboratory because there was no budget to hire people. So the postdocs had gone back to Japan, so a certain period of time I was totally alone. I had no technician, no postdocs.

At the time I noticed that Klotho might be involved in the insulin pathway. So what I did was, I made the recombinant Klotho protein in insect cells; I just injected the Klotho protein and measure blood glucose levels, and indeed, Klotho protein can affect insulin. So that was the first breakthrough of the Klotho protein function.

MEJIA: I want to pursue the scientific breakthrough. I'm just curious at this point, why did you hire all Japanese postdocs?

KURO-O: Well, right. I wanted to hire, of course, good postdocs, but as you know, good postdocs want to go to a better laboratory. It's natural, because if I were the postdoc, I would never go to a laboratory like my laboratory. It's really small, and still struggling to establish my position. Anyway, the direction wasn't clear at that time. So, yes, there were lots of potentials in those mice, but still, until I can show some directions, maybe no postdocs would like to work with me, and I also didn't want to hire postdocs until I could show a clear direction to them. I really wanted to get a good person, but nobody would join my laboratory at that time. It was really tough.

MEJIA: So it sounds like through this whole period, then, you were basically the intellectual driver, you were the person coming up with hypotheses.

KURO-O: Yes. Right. Right. And in addition to the experiment, I had to maintain my mouse colonies and make Klotho protein, basic daily routines, and paperwork, still maintain the laboratory and everything. I had to do it by myself. I couldn't spend 100 percent of my time on

the experiments, so that was also a stress for me. Still, I think it was okay anyway. Somehow I survived.

MEJIA: Do you have any teaching responsibilities here?

KURO-O: Yes, but the teaching duties were very small, and I just had a class for aging, elective courses and just give a lecture once a year. That's all. That was good for me.

MEJIA: What year was it when you were alone in the lab and started to notice the insulin response? Was that 2001?

KURO-O: It was maybe around 2001. Yes.

MEJIA: So that was right around when you got your first yes from the NIH, too. Right?

KURO-O: Right. Based on that very preliminary data, I could show some direction of the Klotho protein function. That helped me to get NIH RO1. So that was really a big breakthrough. Yes, actually, the program director in the NIH was very interested in the Klotho project. When my application got a good score, the program director immediately called me and said congratulations to me. He said that he was very interested in this Klotho project and he really wanted me to get funding. So, yes, he was also pleased to know that I got a good score.

But anyway, from 2002 I got $250,000 every year, so that helped me a lot, of course, and I could get postdocs, technicians, and I could pursue the Klotho protein function on the insulin signaling pathway.

MEJIA: How does your salary work here after the grant runs out? Do you fund yourself?

KURO-O: Yes. Right. Before I got the NIH funding, the department 100 percent paid for my salary. But after I got NIH grant, I replaced a little bit, part of my salary, maybe 20 percent or something. Actually, I got another RO1 two years ago, so then another 20 percent or something like that. So currently I think I pay 40 percent of my salary and 50 percent from the state. So 10 percent from the department, something like that, I think.

MEJIA: I'm curious because that varies a lot depending on the type of institution, it seems like. I'm sorry, I didn't mean to sidetrack you too much from the science, because it was getting

pretty interesting. So you got the preliminary results in that period on your own. You got money. Could you maybe describe the next step? Were did you find your postdoc or technician? How did you go towards gearing up now that you had more of a direction for your proposal?

KURO-O: I found that the Klotho is involved in the insulin pathway, maybe 2002 or somewhere in that. Actually what I first started when I came here was to set up the mouse colony for a lifespan monitoring of the Klotho mutant mice. So you might remember that when I published the *Nature* paper, one of the biggest criticisms was whether the Klotho mutant mice are indeed aging or not. Klotho mutant mice indeed look like aging, but they might just be sick and they just simply develop many diseases, so Klotho mutant mice might not be aging. The *Klotho* gene might not be involved in the natural aging process. That was major criticism. I think that was very reasonable criticism.

So to argue against that, I needed to prove the opposite. If you overexpress Klotho, then the animal would live longer than wild-type mice. If we can show that, that would be the definite proof that Klotho is indeed involved in the aging process, because lifespan extension cannot be explained without assuming that the aging process was inhibited. Lifespan can be shortened by any reason. If the animals are sick, they will die early. It's not necessarily because of aging, but because of diseases.

But the opposite never happens because of disease. The lifespan cannot be extended by diseases. So if the lifespan is extended, then that would directly mean that the aging process has been suppressed. But, as you know, it takes time. Laboratory mice live usually two years on average. So to prove that Klotho overexpres sing transgenic mice live longer than wild-type mice, probably you have to wait three years, four years.

So the first thing I did was to set up the mouse colony for lifespan study. I had transgenic mice that overexpressed Klotho, so I just set up several mice, just kept them, and observed how long they lived. The lifespan data, I finished the lifespan study around 2002. I came here in '98 and set up the lifespan study and waited three or four years. So the lifespan study finished around 2002. It turned out that Klotho overexpression indeed extended the lifespan of the mouse. That was another breakthrough. So two breakthroughs came along at the same time.

MEJIA: So you set that one up to run in the background?

KURO-O: Yes.

MEJIA: To run in the background, that just means to let that one run while you pursued the other.

KURO-O: Right. Right. Sure.

MEJIA: So you created another mouse that overexpressed Klotho?

KURO-O: Right. Actually, I made it in Japan to rescue the Klotho mutant mice. I brought those mice here. So in 2002, I knew that the Klotho is involved in insulin pathway and Klotho overexpression extends lifespan of the mouse.

At that time, studies on lifespan were done usually by using worms and flies, *C. [Caenorhabditis] elegans* and *Drosophila,* because their lifespans are short, so it's easy to monitor their lifespans. Of course, the genetics are very excellent in those model animals. So from the studies on worms and flies, it had become evident that inhibition of insulin signaling pathway can expand the lifespan of worms and flies. That was the emerging concept at that time, and actually that fit in with the Klotho protein function very well, because Klotho can inhibit the insulin pathway and Klotho expression extended lifespan. So the same story might be true in mice. That makes a lot of sense, so probably that's why NIH funded me, that that project might be promising.

So what was the original question? I am sorry.

MEJIA: The original question was about hiring postdocs, but this is good if we can stick with this for a minute.

KURO-O: I'm sorry.

MEJIA: No, this is great. This is really interesting. So okay, I was just checking, it was in 2002.

KURO-O: Okay. So about the postdoc.

MEJIA: Just to make sure I understand, it sounds like—and I do want to come back to finding the postdoc—the model of insulin resistance extending life existed only in the simpler systems. At that point nobody knew for sure. So that was once again a hypothesis.

KURO-O: Right.

MEJIA: It seems logical, but it was a hypothesis that the two were connected.

KURO-O: Right. Exactly right.

MEJIA: Okay. Well, maybe it is a good time, then. So you have good data, you have money, you can take a deep breath. What do you do next?

KURO-O: Although I was funded from NIH, but I needed to really get a good publication. Actually, there was no big publication at that time for me. I found that Klotho was involved in the insulin pathway. I found that Klotho extends lifespan. So actually, I wrote a paper using that data. That paper describes about Klotho overexpression extending lifespan in mice. That fact alone is very interesting, I think. But in addition to that, I showed that Klotho is involved in the insulin signaling pathway, and I combined this data and submitted the paper to *Nature* first, but *Nature* rejected that paper. It was not clear what was wrong in the review process, but anyway, they didn't like my paper.

Klotho can inhibit the insulin pathway. Klotho expression causes insulin resistance, so usually insulin resistance is directly related to diabetes. Usually diabetes won't extend the lifespan. That's bad for health. So that was a major question. But I think it depends on how strong the insulin signaling pathway is. What genetics in lower animals tells us is moderate inhibition of the insulin signaling pathway extends lifespan, but if you inhibit it too much, of course they are not happy, and of course in mammals they develop diabetes. So that's not good. But it depends on how strongly they inhibit the insulin signaling pathway. But such discussion cannot be acceptable for reviewers, and anyway, that paper was rejected.

The next question is, what is the mechanism by which Klotho inhibits the insulin pathway? Because Klotho is a membrane protein, and actually it's expressed only in the kidney and brain, if you disrupt the *Klotho* gene, all organ systems have some changes, aging-like changes. So how do we explain this? Because its membrane protein and expressed only in the kidney and brain, so how can these proteins can affect, say, lung, muscle, liver? So our hypothesis was, the Klotho protein is shed and secreted into the blood and saturated in the blood and targeted on the, say, lung, muscle. That was our hypothesis. So maybe the reason why *Nature* didn't like my initial paper was because there was no explanation about the mechanism by which Klotho inhibits the insulin pathway or extends lifespan. So that's why we tried to purify the Klotho protein, soluble Klotho protein, or make the equivalent protein in the recombinant protein, and purify it and look at whether this protein is indeed active or not. We thought about that hypothesis several years ago.

So I tried to make recombinant Klotho protein, but the Klotho protein is a really difficult protein, and I couldn't get enough amount of recombinant Klotho protein. I tried many different methods, but no method worked very well. That was another, actually, breakthrough. So it was a really difficult protein to make, but somehow I was successful in purifying the Klotho protein. We could do some experiments using that protein, like injecting that protein directly into the

mouse or appling the protein on the cell or something like that, and we could somehow show that this soluble form of Klotho protein is indeed active protein and indeed circulates in the blood and could function as a hormone-like substance.

MEJIA: So this is work that came after *Nature* rejected the paper?

KURO-O: Well, we were working on that, but at that time the data was very primitive primarily, so we just used the conditioned medium of the recombinant protein. That's actually not very good data. We really needed to purify the protein. The method for purification was really tough, and we spent a lot of time purifying the recombinant Klotho protein. But anyway, finally we could do that and revised the paper. Maybe not revised it; it's a completely different paper. We added the data about the recombinant Klotho protein and again applied to *Nature* first. But *Nature* handled our paper as a revision of the previous one, so they sent our paper to the same reviewer, and they again rejected the paper, actually.

Is that okay?

MEJIA: Can I change the tape real quick?

KURO-O: Sure. Sure.

[END OF TAPE 2, SIDE 2]

MEJIA: This is March 7th, and this is Robin Mejia, still here with Makoto Kuro-o in his office at U.T. [University of Texas] Southwestern [Medical Center], the second tape of the March 7th Pew [Scholars Program in the Biomedical Sciences] oral history interview.

So you were telling me about submitting to *Nature*, the first round of rejections. Did you submit it anywhere else, or were you determined?

KURO-O: Well, no, I don't care about the journal, but I believe that that paper was, I think, very interesting, because it was the first example, again, for the lifespan extension in the mouse simply by overexpressing a single gene. It could be very interesting. Because I didn't have any good publication for four or five years, I really need a good publication. Anyway, I wanted to publish in a very good journal, and fortunately, I resubmitted the paper to *Science*, and they accepted it.

Well, as I said, the concept that inhibition of the insulin pathway extends lifespan was

not very acceptable for all people, as I said.

MEJIA: Can I ask you, because it sounds from your description, is this the paper that came out last summer?

KURO-O: Yes.

MEJIA: Okay. Because I did read that one. It's quite interesting. Which was 2005.

KURO-O: Five years.

MEJIA: So you spent another couple years, then, and you were publishing during this time. I have your CV [curriculum vitae]. You were publishing a good bit.

KURO-O: Well, the paper before that was the result of collaboration. Of course, we contributed the data, but those papers were done in both laboratories. My laboratory contributed to that paper, but my collaborator also contributed a lot to that paper. So it's the result of collaboration. But the *Science* paper was really originated from our data, and it's a purely made-in-my-laboratory type of paper. So that was very important for me.

MEJIA: So you got your first results around 2003, 2002, then, from what you're saying and just went back and kept refining?

KURO-O: Yes. Right.

MEJIA: I'm sorry if I cut you off when I changed tape. You were basically explaining that you purified the protein. Did that help you better understand the insulin reaction?

KURO-O: Yes. Right.

MEJIA: How did that tie back together?

KURO-O: To establish the concept that Klotho protein functions as a hormone-like substance

involved in the insulin signaling pathway, we really needed a purified protein because we needed to know whether this protein did bind to the cell surface receptor. Any activity assay or any binding assay required the purified Klotho protein. We actually made the Klotho protein in the insect cell system, but purification was really tough. So that was a major drawback of the Klotho research until 2004 or something like that. But once we got the purified protein, we could do all necessary assays and experiments to prove that the protein indeed functions as a hormone-like substance. So that was not a huge but a very necessary breakthrough for me.

MEJIA: Can we step back a little bit from the science? In a way, it sounds almost like you were not setting up your lab again because you had your equipment and space, but you had your set of Japanese postdocs [postdoctoral fellows] and not so much work. They left. It was a period on your own. Who did you bring in? How did you go about doing the science this next period?

KURO-O: There are many Japanese postdocs working in the U.T. Southwestern, and after I got funded from NIH [National Institutes of Health], I really wanted to hire a very good postdoc. First, I looked inside of U.T. Southwestern for somebody who might be looking for a postdoctoral position, and at that time, one of the Japanese postdocs wanted to move their laboratory and he contacted directly to me. He said he wanted to move anyway.

Actually, I have currently three postdocs. Actually, two of them are promoted to instructor right now, but all of them used to work in other laboratories at U.T. Southwestern, and for various reasons they had to change laboratories. So they were looking for a new laboratory to work for.

And actually I had an ad put in *Nature* or *Science* looking for an available postdoc. I'm not stuck on Japanese guys, but it's just a coincidence that some people wanted to move their laboratory for various reasons, for family reasons, or some people had a little complicated boss, or something like that. But they are really experienced postdocs and they are all Ph.D.'s, so they are very much determined to get their own papers published, and they really work very hard, and I don't need to teach pipetting, how to pipette. Rather, they are much better than me in carrying out various types of experiments.

So yes, I was lucky that I could get good, experienced postdocs by accident, actually. Actually, the two postdocs I got just after I got funded by NIH, so they are very experienced and they had techniques that I didn't have. I'm very good at mouse genetics and transgenic mice, something like that, because I learned a lot about that, but those two postdocs [Hiroshi Kurosu, Masaya Yamamoto] are very good at protein chemistry or signal transduction analysis that I had never done before. So that was really the best match for me. I do what I can do and they do what they can do, and they are really complementary to each other. After I got these two guys, the work progressed very well. Yes, that was a major progress in my laboratory.

Recently, another postdoc [Yasushi Ogawa] joined my lab, and he's an M.D./Ph.D. and he is a dermatologist, actually, and he also has some reason he had to move the laboratory. So

anyway, I got him last year, and he is also working very well. Because of this paper, I promoted them from postdoc to instructor, so they are now instructors here.

MEJIA: So are they still in your lab, or do they have their own?

KURO-O: Yes. They are working with me.

MEJIA: And bringing them in, did that help shape your research at all? Did they bring in ideas?

KURO-O: Yes. For this particular paper, the idea was already there, and they provided the very nice expertise to prove that hypothesis. They are very talented.

Anyway, after publishing this paper, we needed to go to the next step. The next step is to identify the Klotho receptor or Klotho binding partner. That was a really challenging project again. Actually, one of the guys, Dr. Hiroshi Kurosu, the first author of this paper, he actually brought in a new idea. My laboratory is currently changing direction a little bit because of his great findings. He found that Klotho protein binds to the fibroblast growth factor receptor. It's a little bit of a scientific thing. Anyway, he found another protein that interacts with Klotho protein. So, yes, we have been looking for that kind of protein, Klotho interacting protein, for a long time, and he eventually found the receptor for the Klotho protein.

So actually, Klotho protein has a lot of functions, a lot of different functions. So in this paper we described about the involvement of Klotho in the insulin pathway. But Klotho is also involved in regulation of mineral ion homeostasis, so Klotho protein can regulate phosphate homeostasis or calcium homeostasis. Klotho can regulate blood levels of phosphate and calcium. That's a totally new function of the Klotho protein.

I'll tell you about how he found out the relation between Klotho and FGF [fibroblast growth factor]. So I said that Klotho mutant mice show an aging-like phenotype, okay? And maybe two years ago, another knockout mouse was developed and it was reported that they developed aging-like phenotypes. Hiroshi [Kurosu] compared the phenotype of Klotho mutant mice and the reported aging-like mice, and he found that these two were almost identical. The gene knocked out in the reported paper was FGF23, fibroblast growth factor 23. These two mice are exactly the same, not simply resembling, but almost identical. We were really surprised by this fact, because at the time nobody knew that these two genes were related at all. If you knock out FGF23, mice get this aging-like syndrome similar to Klotho mutant mice. FGF23, the name shows it's a fibroblast growth factor which binds to the fibroblast growth factor receptor. FGF is one of the oldest growth factors identified and very well characterized. As you can notice, there are many FGF genes. So 23 means the twenty-third identified.

Because of the similarity of these two mice, he [Hiroshi Kurosu] tried to prove that

Klotho is involved in the FGF signaling pathway. He indeed could show that Klotho can directly bind to the FGF receptor and regulate FGF23 binding to the FGF receptor. It's a kind of co-factor that regulates FGF signaling. This actually makes a lot of sense, because FGF23 was first identified as a factor that inhibits phosphate metabolism in the kidney. So if FGF23 is excessive, and actually there is a disease that produces more FGF23 in humans, it's a hereditary disorder, but such patients show phosphate wasting from the kidneys because FGF23 inhibits phosphate reabsorption from the kidney, and they develop rickets, osteomalacia, and bone diseases.

So FGF23 was a very new, recently identified hormone that inhibits phosphate uptake in the kidney. In the kidney and bone research field, FGF23 was really a topic in the past one or two years, and Klotho suddenly gets into the spirit, and Klotho is a critical factor that regulates FGF function. So actually, Hiroshi and I published the paper about the interaction between Klotho and FGF23, and it will appear in a *JBC* [*Journal of Biological Chemistry]* accelerated publication this week.

MEJIA: Oh, I don't have that one, then.

KURO-O: I'll print it out and give it to you. It's really a hot topic, actually.

MEJIA: That is fast, because you just had two papers. No, maybe that's this one. So you've had two papers in the *Journal of Biological Chemistry* since the *Science* paper.

KURO-O: Yes. Right. The one is about the follow-up of this study. One of the *JBC* papers showed what happens after Klotho inhibits the insulin pathway, after Klotho increases resistance to oxidative stress, or something like that. That's a follow-up of a study. But this opened up a different area of the research, so I'll print out this paper and give it to you.

MEJIA: Thank you.

KURO-O: It will appear March 10th, so this Friday.

MEJIA: They did accelerated publication online?

KURO-O: Yes. I think it's already available.

So we have been working on Klotho and the insulin pathway, but another research

direction appears very promising. This is the Klotho protein function in the FGF signaling pathway. If Hiroshi hadn't noticed the similarity between these mutant mice, then we couldn't notice this fact. It was really lucky for me to have him here, and actually, that is exactly what I wanted. They are very talented postdocs. I am already forty-six, and my brain's getting a little bit stiff, and maybe I need some fresh way of thinking or fresh viewpoint to look at Klotho. I looked at Klotho only from the aging and insulin pathway thinking, but this brought me a very different viewpoint about the Klotho protein to look at.

Currently I'm very busy because of this paper, because FGF23 was really a big topic in the kidney disease field and also in the pediatric research field. There are a lot of mysteries about FGF23, because people didn't know about Klotho's involvement in this pathway. So FGF23 is actually a very difficult hormone. If you just add FGF23 on the cultured cell, there is no activity at all, but if you inject FGF23 in the animal, it is very active. So people assumed that there was something missing in the cultured cells that was essential for FGF23 to be functional. We identified that missing factor was Klotho. So if you inject the FGF23 mice, there is Klotho expressed in the kidney. That's why FGF23 can be functional. But in cultured cells, no cultured cells express Klotho, so even though they applied a huge amount of FGF23, there was no activity at all. People wanted to know why this happened. So Klotho, just adding Klotho, is necessary and sufficient to induce FGF23 activity.

MEJIA: In cell culture?

KURO-O: In cell-culture systems. This is about that. This is actually very interesting because it can be directly applied to patients' treatment. Many patients with chronic kidney disease will die because of vascular complications, like vascular calcification. The biggest problem in the management of chronic kidney disease patients is how to reduce the risk of death of such patients. One of the major risk factors for the chronic kidney disease patient is vascular calcification. Actually, the Klotho mutant mouse shows extensive vascular calcification and could be a very good model for vascular calcification caused by chronic kidney disease. So we are currently trying to see whether injection of Klotho protein—maybe that would be the direct way—might affect vascular calcification. That's a final goal. So Klotho protein might be applicable to treatment of chronic kidney disease if Klotho protein can indeed reduce vascular calcification.

You can inject FGF23 as well, but in chronic kidney disease patients, the kidneys are disorganized, and Klotho protein production is very much reduced in the chronic kidney disease patient. So it's possible that FGF23 injection alone may not be very effective because there is no Klotho produced in the kidney. In that case, we need to add Klotho to remove the phosphate from the body, or vascular calcification.

There are lots of potential therapeutic applications in Klotho. Vascular calcification is really a very difficult disease, and no mechanistic insight was available at that point. Klotho may provide some clue to understand the molecular mechanism of vascular calcification. There

are many expectations of that kind of stuff.

MEJIA: Well, that's real interesting. Because I did give you a heads-up that I would be about two hours, this might be a good time to break, and I could actually read the paper this evening, too, if you want. Does that make sense?

KURO-O: Okay.

MEJIA: Because this is a really interesting area, so I can read this.

KURO-O: Yes, it's another area of Klotho research that just recently came up.

MEJIA: Okay. Well, maybe then we can pick up with this.

KURO-O: Okay. Yes. I'll go and get the paper.

[END OF TAPE 3, SIDE 1]

[END OF INTERVIEW]

INTERVIEWEE: **Makoto Kuro-o**

INTERVIEWER: **Robin Mejia**

LOCATION: **University of Texas Southwestern Medical Center**
Dallas, Texas

DATE: **8 March 2006**

MEJIA: Hi. This is Robin Mejia here with Makoto Kuro-o in his office at U.T. [University of Texas] Southwestern [Medical Center] on March 8th for the third and final day of the Pew [Scholars Program in the Biomedical Sciences] oral history interview.

Good to see you again this morning.

KURO-O: Good morning.

MEJIA: When we finished up yesterday, you had been talking about getting to the point with Klotho research where you had demonstrated that what was going on was really aging by growing mice that overexpressed and then living longer. Then you had also started to explain a new direction in your research that had been picked up on by one of your postdocs [postdoctoral fellows], the instructor Hiroshi Kurosu. I just took a look at that paper last night. That was pretty interesting. Are you going to be pursuing any new studies along these lines?

KURO-O: Yes. Right.

MEJIA: Can you talk a little bit about where that will go?

KURO-O: Okay. I think Klotho protein has a lot of functions. Klotho protein is a multi-functional protein, maybe. Inhibiting the insulin signaling pathway is probably only one of those multiple functions. Actually, very recently another group published a very interesting paper about Klotho. They are working on some kind of ion channel expressed in the kidney called TRPV5 [Transient Receptor Potential V5]. . This channel is very important in calcium reabsorption in the kidney. They reported that the Klotho protein can activate this channel through its enzymatic activity.

Actually, Klotho protein has a homology to an enzyme called glucosidase. It was extensively investigated in plant biology or bacteria. It is expressed in bacteria, in plants, and

there are lots of family members in glucosidase. Klotho protein has a weak homology to this enzyme. Klotho protein does not have enzymatic activity like glucosidase but has a little different type of enzymatic activity called glucuronidase. These enzymes hydrolyze sugar chains, any kind of sugar chains. The surface protein is usually decorated by sugar chains, and those sugar chains are very complicated. But recently, those sugar chains are known to affect renal function or protein function. So in their paper, they claim that Klotho modifies sugar chains on the TRPV5 ion channel, thereby regulating its activity.

It's a very new concept, actually, so that's why it's published in *Science* right after our paper. We didn't notice this fact, but this is really an interesting finding, because we thought that Klotho functions as a hormone-like substance. It's also true in this case because they claim that the extracellular domain or soluble form of Klotho protein can directly impact with this ion channel and chew up some decorated sugar chain, modify the sugar chain, and regulate TRPV5. So it's a very new concept, that an enzyme can modify protein function through changing its sugar chain. It's a very new concept, actually. Klotho may be the first example of this kind of regulation.

Klotho can function as a hormone-like substance. It can also function as an enzyme, and it can also function as a co-receptor or co-factor that is essential to fibroblast growth factor-23. There are many faces of Klotho. We don't know if these functions are related or if these are completely independent functions of the Klotho protein. We don't know at this point.

There are many ways to approach the Klotho protein function. One of the ways we propose would be to look at the effect of Klotho on the fibroblast growth factor signaling pathway. That's also probably an important aspect of Klotho protein function. We can try many experiments to look at Klotho protein function.

MEJIA: You think Klotho will keep you busy?

KURO-O: Yes. Yes, I think so. The new concept [for regulation of TRPV5 ion channel and calcium ion concentration] they proposed is really, I think, interesting.

MEJIA: Are you talking to that group?

KURO-O: No, not directly, but I'm collaborating with the investigator here at U.T. Southwestern who is working on this kind of ion channel, and actually we're getting very interesting data, but I cannot yet publish it. We are still collecting the data. But it's really interesting. So Klotho is indeed a multifunctional protein, and there are a lot of things to do.

MEJIA: Can you maybe talk for a minute, too, on the bigger picture? Between 1997 and,

really, last September you had worked largely independently or with your own postdocs collaborating here, but probably not many other people outside your school knew that much about your work at the time, or your results. Then you published in a very major journal in September that's read all over the world. Scientifically, what the reaction?

KURO-O: Yes. Actually, I cannot handle with all the reaction. Of course, there were some people working on insulin signaling in the diabetes field who were very interested in Klotho protein function. That's very natural. But what I was surprised at was the people who are working on kidney disease or phosphate/calcium metabolism or the bone-metabolism field were also very interested in Klotho, because they knew about Klotho mutant mice, which I reported in 1997, back in 1997 in *Nature.* They looked at the phenotype and found that they developed calcification in vessels and in the kidney. So they were very interested in this phenotype. Of course, I didn't know anything about the mechanism, why Klotho deficiency causes such calcification, but now one of the Klotho protein functions was revealed last year, and in that *Science* paper I proposed that the Klotho may function as a hormone-like substance, so that makes the researchers in the kidney field think that Klotho may function as a hormone that prevents vascular calcification or blocks kidney calcification.

And almost at the same time, the FGF23 had been identified, and of course, they are also very interested in FGF23, and this paper linked nicely the Klotho and FGF23 stories. After the publication of this paper, more and more kidney people are asking me to work with them. And actually I think it's getting to be a very exciting topic in that field. So that's probably the biggest reaction and from two different research fields, diabetes and insulin-pathway field and kidney disease and bone-metabolism field. Those are the two responses.

MEJIA: So are you hearing from researchers in, maybe, projects that you didn't even know were happening?

KURO-O: Yes. Actually, I have a couple of collaborators here in U.T. Southwestern after or just around when I published the *Science* paper, and yes, so they are, of course, interested in phosphate metabolism and ion-channel-activity regulation by the Klotho protein. So that's very nice.

MEJIA: That actually kind of brings up an interesting related point. Do you know how many different labs there are at U.T. Southwestern, roughly?

KURO-O: I think several hundreds, maybe. There are lots of laboratories, and some laboratories have a number of PIs [principal investigators], but the number of independent faculty members should be around one thousand maybe. It's a big institute.

MEJIA: I asked you partly because you've mentioned a couple of times that you have internal collaborations.

KURO-O: Yes. Right.

MEJIA: And those are mostly biomedical researchers of one type or another?

KURO-O: Right.

MEJIA: So you're probably in an unusual position in this community, in being able to find a lot of expertise without having to go very far.

KURO-O: Yes, indeed. I'm really taking advantage of this community. The U.T. Southwestern community is really great. I can find any collaborator. If I have any questions, I can find people I can ask. That's really an amazing thing.

Maybe if I were in Japan, I could not get this kind of good community. Of course, Japanese science is really good, but the number of investigators in the similar community or similar institute is probably around one hundred or two hundred at the most. Even the biggest university, like Tokyo University, the community is not as big as here. Yes. So anyway, it's Texas, yes? Everything is very big. I think it's a very good thing.

MEJIA: Is it competitive at all?

KURO-O: Well, actually I'm very surprised. All collaborators are very easy to communicate with, and we can discuss very freely. We can exchange very preliminary data and published data in a very effective way. There is no sectionalism at all. So I am very surprised on that point.

But anyway, we are working as a team, and so any interesting data found in any member of the team, the group or team can share when it's by anybody and develop another hypothesis, another way to experiment. So yes, I think it's very good. I think we are making a very good research team on Klotho protein function and on kidney function or phosphate metabolism. That's very good.

MEJIA: Let me just flip back here, if you don't mind, for one sec.

KURO-O: Sure.

MEJIA: Well, the other questions I wanted to ask: You have a largely research position, but you're in the university, are you on a fairly traditional tenure track here?

KURO-O: Yes, I'm on the tenure track, actually I will be promoted this September to tenured associate professor. Otherwise, I might have to go back to Japan. But I can continue the Klotho work here.

MEJIA: Yes. I'll bet they want to see you continue it here at this point.

Well then, that kinds of leads me to more of a big-picture kind of question. You mentioned yesterday that as you become a professor, you take on more responsibilities than just getting your science done.

KURO-O: Right. Right.

MEJIA: Can you kind of talk about how that's changing? Are you going to be teaching more?

KURO-O: Yes, right.

MEJIA: Do you review papers for journals or do study sections or anything?

KURO-O: Yes. Probably one of the most important works as PI is to get enough grants to run the laboratory. Of course, there are many other things to do, to provide a good research direction and research topics for postdoctoral fellows or students. Of course, there are some teaching duties and much paperwork to maintain the laboratory and many such kinds of stuff. But all those things would be very easy if we are doing good science; because if we have good research topics, we can get grants and I can provide good research topics to the postdocs, and everything will be working very well.

In these two years, I found a couple of interesting research directions in Klotho. So I believe I'm doing pretty good science right now. Currently I'm a little bit optimistic about the future, about getting grants or getting good people. But it's only this year or last year. Before that, I was really worried about what direction we should go, and I was always asking am I doing good science or not, or something like that. But in these three years, I think, I can get

good collaborators, and I can see a couple of very promising research directions. So in that sense, I think it's okay, it's okay.

But I am getting a little bit busier arranging collaborations or doing more grants writing, and maybe teaching duties will be increasing in the next year. I may not spend a lot of time to the research itself, but I think it's the natural way to expand my laboratory a little bit.

MEJIA: That makes sense. At this point, have you still been involved sort of day to day in doing the science at the bench? Are you still at the bench?

KURO-O: Oh yes. I'm working at the bench, recently maybe 50 percent of my time. So three years ago, I spent 90 percent of my time at the bench making data and maintaining mice. My laboratory is still very small. I have three postdocs, and that's it. So actually I'm the only person who works on the mouse colony. I maintain all the mouse colonies and make recombinant Klotho protein. My basic idea is, what I should do at the bench is probably provide good material and good reagents. The postdocs can use those reagents and get interesting data or do the experiments.

I think a small laboratory might be similar to a small restaurant, actually. So maybe the owner—that's me—should get good ingredients from the market and get good decoration of the interior or setting up all the tables. The postdocs should be the chefs. So then I can provide great materials, and then they will make very nice dishes. Then the restaurant will run very nicely. That is maybe similar to running the laboratory, small laboratory. Maybe some of the postdocs are very good and can make better dishes than myself. In that case, maybe I don't need to worry about that, just give them the good material. Then they will cook very nicely.

My role in the laboratory is changing. At first, I just behaved as a chef, and I needed to make the dish by myself. But if the laboratory is getting a little bit bigger, I can hire good chefs, and I can just leave them everything and just arrange the environment so that they can work competently. That would be probably the best way to run the laboratory.

MEJIA: With the paper we were talking about earlier and with the fibroblast growth factor work, did he [Hiroshi Kurosu] bring the idea to you, and you guys discussed it, and you said, "Go for it"?

KURO-O: Yes. Right. So the first thing that Hiroshi noticed was the similarity between the phenotypes of the Klotho mutant and FGF23 knockout mice. That was the beginning of this idea. So of course we discussed a lot what kind of experiment we should do first. But it's pretty straightforward. If two mutant mice show an identical phenotype, then these two genes must be related to each other. The question is which is upstream and which is downstream or something like that. Or they might be working together.

MEJIA: How does it work when you get to the point of publishing? Who wrote the paper?

KURO-O: I wrote the paper. I'm a little bit more experienced in writing than Hiroshi, who was the postdoc. That's why I wrote it. I got informal information that some groups in Japan might have noticed a similar thing. I didn't know how much they knew, but we needed to hurry up. So we worked effectively together. He did the experiments, generated the data, and I was writing the paper and organizing the other data and what data is missing or something like that. We interacted extensively, and all the lab members helped him to get the data. Actually, this was a really quick paper. It took only, maybe, three or four months to get all the data from the beginning to the submission. It was pretty quick. Yes, it was a really nice interaction with the postdocs and myself, and they did the things they are good at.

MEJIA: What kind of hours do you work now? From morning till night. What's a day like? Walk me through a day.

KURO-O: I'm an early bird. I'm in around seven-thirty and work about eleven to twelve hours and leave the office around six-thirty or seven-thirty. It was much better than when I was in Japan, you know? I worked sixteen hours when I was in Japan. But I'm getting older, so twelve hours, eleven hours would be more than enough, maybe. But as I said, my laboratory is still very small, so there is lots of work to do, daily work to do.

MEJIA: Can you maybe describe a typical day? You arrive at seven-thirty. Then what do you do?

KURO-O: Maybe I usually spend time doing experiments or taking care of the mouse colonies. I'm getting a lot of e-mails about collaboration, so I spend most of the time in the morning communicating with other investigators or arranging a collaboration or reviewing new data.

In the afternoon I usually do some experiments. My duty in the experiments is to purify recombinant Klotho protein and provide the protein to my collaborators or, of course, my postdocs and to maintain the mouse colonies and provide mice to collaborators and postdocs. Or sometimes they need tissues of the mice, and then I do the dissection and provide it to them, something like that.

It's very different from day to day. Some days I have to spend most of my time in the laboratory. Just before the grant due date, I have to spend almost all the time here, writing up the grant. It's quite flexible.

Actually, I just completed continuation of my RO1s last month. So last month was really hectic. I had to spend most of my time writing up the grant.

MEJIA: So you finish up at six-thirty or seven-thirty?

KURO-O: Yes.

MEJIA: Do you usually just go home for the evening?

KURO-O: Yes. Right.

MEJIA: Do you have any hobbies?

KURO-O: Well, I'm trying not to come to the office on weekends, as much as I can. I try to spend time with my wife [Kumiko Kuro-o], because, yes, weekdays I cannot spend much time with my wife. It's a very normal weekend. I just go shopping with her or just go to see movies. It's pretty normal.

MEJIA: Let me just check. We've got some time left.

I just want to move back to the science again, for a little bit, because I was thinking about it. At the end of yesterday, you were describing how the new Klotho work may tie in to disease applications. Can you give me a better sense. This is still basic research, at this point, right?

KURO-O: Yes.

MEJIA: Is that something that might lead to treatments ten or fifteen years out or three or four years out? Do you have any sense of that at all? Are you thinking along those lines or not?

KURO-O: Yes. So, yes, of course, the long-time goal would be to use Klotho protein to treat, or to suppress, the aging process in humans, but I don't know if that dream could come true in the next ten or twenty years. Or if you could find some small molecular weight mimetics of Klotho protein, then the mimetics could be applied to the treatment of chronic kidney disease. That's probably the one that's the closest application of the Klotho protein to humans.

Before that, a more practical way to use Klotho research to patients' care is to measure blood concentration or urinary concentration of Klotho protein. Klotho protein is secreted in the blood or excreted in the urine. We are currently working on the method to measure blood and urinary Klotho protein concentrations in clinical support. And if you can find any correlation between Klotho concentration and some specific disease conditions like kidney failure or any kind of age-related diseases, then that would be useful for diagnosis or deciding the prognosis of such age-related diseases. So that might be a pretty close goal, because we have almost finished establishing a method.

The next step would be to collect as many clinical samples as possible and measure Klotho protein concentrations in those samples and see whether any correlation might be observed in some renal disease. And the next step is, if Klotho protein is indeed useful in preventing vascular calcification or kidney failure in the chronic kidney disease patient, then Klotho protein itself or Klotho protein mimetics might be useful. That could become true within ten years or so. I don't know.

MEJIA: When you move from the real basic questions into looking at developing a diagnostic or eventually a drug—and therapy with a small mimetic would be, right?— how does the intellectual property work? Do you or the university patent any of these ideas, or how does that work?

KURO-O: If we can find any such compound, then that would be really good intellectual property, but at this point I don't know whether I can find it or not. I don't know very well about how the patent works or how intellectual property works, but if that helps me do more basic research, that would be great. If the university can get some advantage because of my Klotho research, that would be very great. I got a lot of things from the U.T. Southwestern community, so if my research is useful for the U.T. Southwestern community, that would be great. But at this point I have no idea.

MEJIA: Okay. It's still early for that.

KURO-O: Just doing basic research right now.

MEJIA: Do you do any consulting at all?

KURO-O: No, I don't. I just sometimes receive journal reviews. That's all.

MEJIA: I'm just curious, because actually, among the researchers I've talked to, most are basic

researchers, whereas you can see a long-term application. From the last thing we talked about yesterday, it sounds like you could potentially have some.

KURO-O: Yes. Right. It's still a potential, maybe. I've been contacted by some pharmaceutical companies, but still the Klotho research is basic research, and it still has a long way to go to apply to developing drugs and medicine. So, yes, they are interested; the pharmaceutical companies are interested in Klotho because their research interest is similar to my research interest. Getting some drugs or developing some new drugs, it's maybe a little too early to discuss about that. We still need to know what the Klotho protein is doing right now.

MEJIA: It sounds like one challenging thing about Klotho protein might be the fact that it does so many things.

KURO-O: Right. Exactly. Maybe we are looking at the Klotho protein from a different angle. Klotho inhibits the insulin pathway, regulates the FGF [fibroblast growth factor] pathway, regulates the ion channel. If the enzymatic activity of Klotho is really essential to all these functions, then we can focus our effort to look at Klotho as an enzyme and see what regulates Klotho protein enzymatic activity. At this point, I don't know what is the closest way to reach to the core part of the Klotho protein function. We have several ways to go. That's a good thing.

MEJIA: That makes sense. I think that's not an uncommon route, to find something that's naturally active and see if you can tease apart the pieces. I think it took ten or fifteen years from when people started looking at cannabinoids, and now they've finally got drugs on the market.

KURO-O: Yes. I hope so.

MEJIA: They related the pieces of it.

KURO-O: Yes.

MEJIA: Okay. I think we're ready to change the tape. I'm just going to flip this.

[END OF TAPE 4, SIDE 1]

MEJIA: This is Robin Mejia still here with Makoto Kuro-o on the second side of the fourth

tape.

We just talked about eventual medical applications, and you've mentioned a couple of times today that you feel like you have a handle on several distinct interesting research directions. And I noticed from your CV [curriculum vitae] you have two, or one now, [RO 1 grant]. You mentioned one you just submitted for renewal, and you have other grant ideas. How do you see yourself moving forward in the practical matter? Do you need to recruit more people, or are you going to stick with your lab size? What are you going to do? How are you going to move this all forward?

KURO-O: I want to expand the laboratory, indeed, because there are a lot of interesting topics there. But another way to go is to expand my collaboration in U.T. [University of Texas] Southwestern [Medical Center], and that is, I think, probably the best way to go, because I don't need to get so many new grants to expand my research, I can just talk with other people and expand the collaboration, so they can expand the research using their own expertise. And if I can collaborate with clinicians, they will take advantage of their access to the patient sample for the patient data.

I think Klotho research will expand in various ways, and maybe only a single laboratory cannot handle everything. Maybe my laboratory should focus on the very basic part of Klotho protein function itself, and then I can expand collaboration with faculty at U.T. Southwestern. There are lots of patients visiting this area, so I believe we can do a good clinical study. I'm not so experienced for that kind of study, so maybe I should find a good collaborator and work with him or her.

MEJIA: This would be maybe looking at the diagnostics?

KURO-O: Yes. For example, if I can have access to blood samples or urine samples of chronic kidney disease patients, that would be very interesting. Some doctors may have access to some cancer patients or some lung-disease patients. Anyway, I cannot imagine how many patients are visiting every day here at U.T. Southwestern, but I think maybe this is a very good place to do both basic and clinical research.

MEJIA: Up until now, all your Klotho work has been in mice, right?

KURO-O: Right. Exactly. Right. So in mice, I know how to handle mice, but I don't know what kind of study is possible using clinical samples. Some studies might need collaboration with patients themselves. I don't know how to arrange those studies at this point. Because of this, I really need a good collaborator who will be very familiar with that kind of research design and study. But maybe we can do that. Actually, one of my collaborators is doing both

basic research and looking at outpatients, and he has access to lots of clinical samples. So we are talking about measuring in blood and urine Klotho protein concentration using those samples. So yes, actually the collaboration has just started recently, so maybe we can get some data pretty soon.

MEJIA: Has anyone done that: confirmed that you see the same activity in people that you do in mice, or no?

KURO-O: Well, maybe no, maybe no, because we are probably the only laboratory that can measure blood and Klotho protein concentration at this point. So no laboratory can do that at this point. I don't know about two years later.

MEJIA: Right. Probably more people are looking at Klotho right now than were this time last year.

KURO-O: Yes, I think so.

MEJIA: I have a few more big picture questions I want to get to. You seem to have a pretty solid sense of going forward. If you were going to look back on your career path, reflect, and say you were talking to somebody at the postdoc [postdoctoral fellow] level today, do you have any particular thoughts on things you might do differently, or not, or some advice you'd want to give someone about how to do this?

KURO-O: Well, all right. I don't know. Seriously, I think I did what I could do at each career stage. If other people found the Klotho mouse, noticed the mutant mice that showed aging-like phenotypes, other people may have responded in a different way. Some people might have just thrown them away, or some people might have done much better than me. Actually, my career was influenced by coincidence a lot. As you know, I did not intend to devote myself to aging research. I'm here because I happened to get the mutant mouse. It was an accident. It was just an accident, a coincidence. But my career was influenced, and my present state was not as I imagined when I was a high-school student or I was in the university. But I think it's okay. That's life, maybe.

MEJIA: One thing that came up several times when you were describing your career trajectory was the hours and the amount of time, which is, I think, probably similarly true to people going through the early stages of their career trajectory in the U.S. [United States] as well as in Japan. But it brings up another issue that's been in the news, that a lot of organizations are looking at lately, which is both women and minorities in science. Working in the U.S., you have more

experience with the second one.

KURO-O: Yes. Right.

MEJIA: But a lot of times the career trajectory calls for the longest and the craziest hours right through somebody's late twenties and into their thirties. Do you in particular, or do you know if this institution has any particular thoughts on women in science right now? Do you see very many women coming through towards senior ranks here?

KURO-O: Certainly in the case of the University of Tokyo, my class had one hundred students in one class, and only three of them were women. Actually, it's always like that. So 95 percent or more were male students, and the women were very rare. From the beginning, there was a huge bias, so naturally, all the careers are men.

In a sense, all careers helped women, because, traditionally, in Japan, M.D.'s were all occupied only by men, maybe from more than a hundred years ago, from the beginning of when the modern university system started in Japan.

Actually, I didn't have a chance to think about the minority problem or women problem, because it's too minor. The minority problem will be a problem if a minority group occupies, say, 10 percent of the population or 20 percent of the population. If it's only 1 percent or 2 percent, we rather try to help or protect. There's no official rule or official regulation how to solve the problem about minorities or the gender problem. I don't know. Actually, I don't have any chance to work with women doctors, so I have no idea.

In addition, in Japan there is no racial problem because there is only one, the Japanese race, over there. No foreigners at all. So it's quite an unusual world there. But in that sense, the Japanese have no sense of racism. I really think so because of what I felt when I first came to the [United] States. I was very poor at the English, and I couldn't make myself understood, say, at McDonald's or a hotel. Can you speak Japanese, maybe?

MEJIA: No.

KURO-O: So, if you came to Japan, if you said something, tried to say something in poor Japanese, then Japanese people would try to understand you and try to talk to you in English, even though they are very poor at English. But that could never happen here in the States. When I was using poor English and trying to communicate with some people, usually they never spoke slowly or tried to use easier words, but just, "I'm sorry. I can't understand you." Then they would just repeat the same thing, and, of course, I couldn't understand that. So maybe they cannot understand the people who cannot speak English. So maybe that's a point of

miscommunication. They cannot imagine what is the state of those who cannot understand what people are saying. You know what I mean? So probably that's why they simply thought that I couldn't hear them very well or something, and they would just repeat the same thing. But what I really wanted them to do was to speak more slowly or to just write down what they said. I can read, so once it's written down, I could probably understand. Anyway, so the topic is going in a different way.

MEJIA: No, that's good.

KURO-O: But in that sense, I grew up in a very homogenous society. There were not so many problems about racial difference or minority problems. And of course, I was in an almost 100 percent male world; there were no women, so I had no chance to think about it.

MEJIA: It sounds like, maybe, actually, the cultural bias against women pursuing science careers would be stronger in Japan than here.

KURO-O: Right. Exactly. I imagined that the United States would be a more heterogeneous society, and in fact, there are many women scientists working here, and there are many people from different countries and different cultures. So I think that's sometimes really very stimulating, to know a different way of thinking or to know a different culture. But sometimes, of course, there is some miscommunication, or something I thought would be good for them might not be good for them or something like that. That could happen. But the good thing is, we are doing science, so science is really science. It's good training for me to explain my data or my concepts, so it's easy to understand for any cultural background.

MEJIA: Do you have any female collaborators here that you're working with?

KURO-O: At this point, yes, I have a woman collaborator, not here but outside, in another university. I think we are working very well.

MEJIA: Now, one thing that people have looked at and found is that in the U.S. there seems to be fairly equal representation in most fields up through graduate school and postdoc level, and it's getting into the tenure-track and full professor positions that the drop-off with women tends to happen. The hypothesis is that it's because the most intense period of the career is the same time people have to decide if they want to have kids or not.

KURO-O: Right. Exactly. That's a big factor.

MEJIA: Because if you wait till you're in you midforties and are more settled in your career, you very well likely may have missed your window for children.

KURO-O: Right. Yes, exactly. That's, I think, the biggest factor.

MEJIA: Just to follow up on the other thought, when you came here, did you find that communication was an issue in doing science as well, or mostly in your day-to-day other parts of life? When you were getting established and trying to understand the American system, did that pose challenges at work, too?

KURO-O: Well, actually I didn't have any big problem in learning how the system works in science, because all people knew that I didn't learn about this system so people helped me a lot. Even if I had a very ridiculous question for them, they taught me very well. But I think the system here is very reasonable and understandable. Yes, a similar system actually exists in Japan, but in a sense, the Japanese system is a little bit more bureaucratic. You have to do a lot of paperwork. Sometimes it seems a little bit like nonsense.

But I think the system here is working very well so that we can focus on science. I think the system is very mature here. At first, I just took some time to understand how the system works, but once I understood the system, it was actually very nice. So, I can spend more time, not for the paperwork but for the science. I can spend much more time. I couldn't do like this if I were in Japan, maybe.

In the Department of Pathology, at least, there are lots of people working to support our research, like secretaries and pathology technicians, and the chairman of department has very strong support people, and they actually can take care of most of the nonscience part of my job. So that helps me a lot, indeed.

MEJIA: What's your residency status? Did you apply for citizenship, or are you here on a visa?

KURO-O: I got a Green Card [permanent residency card] last year. It took three years for that route.

MEJIA: That's pretty fast.

KURO-O: Yes. Yes. It's still very fast, I think, yes. So I first got H-1B [visa] when I came here,

and it's valid for six years, and after six years, I had to choose whether I got a Green Card or go back to Japan.

MEJIA: Do you see your research keeping you in the U.S. indefinitely at that point?

KURO-O: Yes, at least for a while. I can survive for a while. I will get tenure. And as long as I can get funded, I can continue working on Klotho here.

MEJIA: Is that your preference at this point?

KURO-O: Well, yes. I just expanded my collaborations here, so I would like to stick here for at least the next several years. But I don't know what happens after ten years or seven years. But I'm very, very satisfied in the current funding situation and collaborators, so I don't feel like moving anywhere at this point.

MEJIA: That makes sense. I just asked because I remember at the beginning you had said you had never intended to even look in the U.S.

KURO-O: Right. Maybe I'm lucky. I didn't see anywhere else, but I think this is very good here for me.

MEJIA: Well, we have actually covered, I think, most of the stuff that I specifically wanted to ask you. Were there other areas? Was there anything that you wanted to address about science or your lab or your students that I didn't think of asking you? The state of science in the U.S. as a whole?

KURO-O: Maybe I should comment a little bit about the difference between the Japanese system and the United States system.

MEJIA: That would be great.

KURO-O: Yes. I remember that the total budget for science spent every year is more than ten times more here in the States when compared to Japan. That's a huge difference. Recently Japanese science is getting much better than before, and the government invests more and more money, in the past maybe five years or so. And the Japanese system is getting better and better

recently. But still, the total budget is less than one tenth. That makes a big difference. And of course, the quality and quantity of the science, or the publication of papers, cannot compare with the United States. Everybody knows that the United States is in the number one position right now. In that sense, it's very lucky for me to be here and work in the States. But I don't know when but in the future, I would like to contribute to Japanese science in some way. Of course, they are two different countries with a different historical background, and there're different budget systems, so it's probably wrong to directly bring the United States system directly to Japan, apply to Japan its system. But maybe what I learned here can be of some help to improve the Japanese system.

So I don't know when it will be, but maybe five, seven, ten years later, I hope I make some contributions to improve Japanese science, the system, because I am Japanese, and my cultural background and my scientific background, all the education, was done in Japan. So I'd like to contribute in some way to Japanese science.

Nowadays, many Japanese scientists work here in the United States and get back to Japan, so I may be just one of them. But we have, probably, a lot to learn from the United States science system. But, that's what I am lately thinking about the long-term future.

MEJIA: That's interesting and makes sense. It's a challenge, probably, because I've heard from other people that have come to the U.S. to do science that it's by far easier to do science here, as so many smart people come from everywhere to do science here.

KURO-O: Right.

MEJIA: That's interesting. So you may yet end up back in Japan.

KURO-O: Maybe. If anybody can provide me a good position there.

MEJIA: Well, no. There's much to said for being someplace where you've gotten research done that contributes a lot to the global scientific understanding. You were able to do it here, so there's a lot to be said for that.

KURO-O: Right. So maybe I can do the same thing, not maybe the same thing, but we can continue Klotho work anywhere, maybe. Anybody can start Klotho work anywhere. Science is like that.

MEJIA: But yes, it helps to be in a place where you're getting it established, though, financial

support and the freedom you've got here.

KURO-O: Yes. Right. It took a lot of time and effort to get the current situation, so I'll be several years here, at least.

MEJIA: Great. Well, thank you so much.

KURO-O: Thank you very much.

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END OF INTERVIEW

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