Regional Oral History Office
The Bancroft Library

University of California
Berkeley, California

Program in Bioscience and Biotechnology Studies

ARTHUR D. RIGGS
CITY OF HOPE'S CONTRIBUTION TO EARLY GENENTECH RESEARCH

Interviews Conducted by
Sally Smith Hughes
in 2005

Since 1954 the Regional Oral History Office has been interviewing leading participants in or well-placed witnesses to major events in the development of northern California, the West, and the nation. Oral history is a method of collecting historical information through tape-recorded interviews between a narrator with firsthand knowledge of historically significant events and a well-informed interviewer, with the goal of preserving substantive additions to the historical record. The tape recording is transcribed, lightly edited for continuity and clarity, and reviewed by the interviewee. The corrected manuscript is indexed, bound with photographs and illustrative materials, and placed in The Bancroft Library at the University of California, Berkeley, and in other research collections for scholarly use. Because it is primary material, oral history is not intended to present the final, verified, or complete narrative of events. It is a spoken account, offered by the interviewee in response to questioning, and as such it is reflective, partisan, deeply involved, and irreplaceable.

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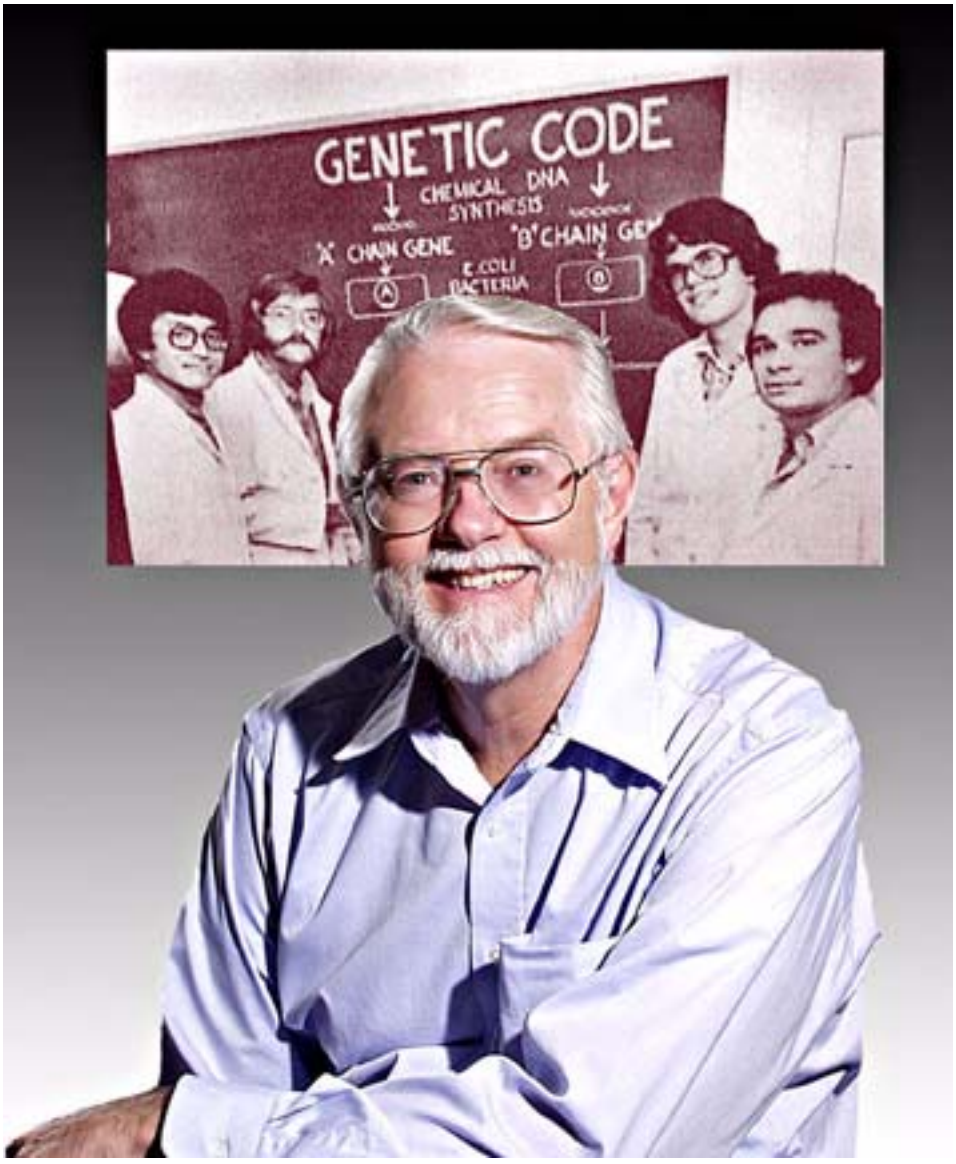

Dr. Arthur D. Riggs. In background photo: Keiichi Itakura, Riggs, David Goeddel, and Roberto Crea, City of Hope/Genentech team that cloned human insulin gene.

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Genesis of the Program in Bioscience and Biotechnology Studies

In 1996 The Bancroft Library launched the forerunner of the Program in Bioscience and Biotechnology Studies. The Bancroft has strong holdings in the history of the physical sciences--the papers of E.O. Lawrence, Luis Alvarez, Edwin McMillan, and other campus figures in physics and chemistry, as well as a number of related oral histories. Yet, although the university is located next to the greatest concentration of biotechnology companies in the world, the Bancroft had no coordinated program to document the industry or its origins in academic biology.

When Charles Faulhaber arrived in 1995 as the Library's new director, he agreed on the need to establish a Bancroft program to capture and preserve the collective memory and papers of university and corporate scientists who created the biotechnology industry. Documenting and preserving the history of a science and industry which influences virtually every field of the life sciences and generates constant public interest and controversy is vital for a proper understanding of science and business in the late twentieth and early twenty-first centuries.

The Bancroft Library is the ideal location to carry out this historical endeavor. It offers the combination of experienced oral history and archival personnel and technical resources to execute a coordinated oral history, archival, and Internet program. It has an established oral history series in the biological sciences, an archival division called the History of Science and Technology Program, and the expertise to develop comprehensive records management and to digitalize documents for presentation on the Web in the California Digital Library. It also has longstanding cooperative arrangements with UC San Francisco and Stanford University, the other research universities in the San Francisco Bay Area.

In April 1996, Daniel E. Koshland, Jr. provided seed money for a center at The Bancroft Library for historical research on the biological sciences and biotechnology. And then, in early 2001, the Program in Bioscience and Biotechnology Studies was given great impetus by Genentech’s major pledge to support documentation of the biotechnology industry. Thanks to these generous gifts, the Bancroft is building an integrated collection of research materials--oral history transcripts, personal papers, and archival collections--related to the history of the biological sciences and biotechnology in university and industry settings. A board composed of distinguished figures in academia and industry advises on the direction of the oral history and archival components. The Program's initial concentration is on the San Francisco Bay Area and northern California. But its ultimate aim is to document the growth of molecular biology as an independent field of the life sciences, and the subsequent revolution which established biotechnology as a key contribution of American science and industry.

Oral History Process

The oral history methodology used in this program is that of the Regional Oral History Office, founded in 1954 and producer of over 2,000 oral histories. The method consists of research in primary and secondary sources; systematic recorded interviews; transcription, light editing by the interviewer, and review and approval by the interviewee; library deposition of bound volumes of transcripts with table of contents, introduction, interview history, and index; cataloging in UC Berkeley and national online library networks; and, in most cases, digital presentation at http://bancroft.berkeley.edu/ROHO/projects/biosci.

Sally Smith Hughes, Ph.D.
Historian of Science
Program in Bioscience and Biotechnology Studies
The Bancroft Library
University of California, Berkeley
November 2005

ORAL HISTORIES ON BIOTECHNOLOGY

Program in Bioscience and Biotechnology Studies
Regional Oral History Office, The Bancroft Library
University of California, Berkeley

Paul Berg, Ph.D., *A Stanford Professor's Career in Biochemistry, Science Politics, and the Biotechnology Industry*, 2000

Mary Betlach, Ph.D., *Early Cloning and Recombinant DNA Technology at Herbert W. Boyer's UCSF Laboratory*, 2002

Herbert W. Boyer, Ph.D., *Recombinant DNA Science at UCSF and Its Commercialization at Genentech*, 2001

Roberto Crea, Ph.D., *DNA Chemistry at the Dawn of Commercial Biotechnology*, 2004

Donald Glaser, Ph. D., *The Bubble Chamber, Bioengineering, Business Consulting, and Neurobiology,* 2006

David V. Goeddel, Ph.D., *Scientist at Genentech*, CEO at Tularik, 2003

Herbert L. Heyneker, Ph.D., *Molecular Geneticist at UCSF and Genentech, Entrepreneur in Biotechnology*, 2004

Keiichi Itakura, *DNA Synthesis at City of Hope for Genentech*, 2006

Irving S. Johnson, Ph.D., *Eli Lilly & the Rise of Biotechnology*, 2006

Thomas J. Kiley, *Genentech Legal Counsel and Vice President, 1976-1988, and Entrepreneur*, 2002

Dennis G. Kleid, Ph.D., *Scientist and Patent Agent at Genentech*, 2002

Arthur Kornberg, M.D., *Biochemistry at Stanford, Biotechnology at DNAX*, 1998

Laurence Lasky, Ph.D., *Vaccine and Adhesion Molecule Research at Genentech*, 2005

Fred A. Middleton, *First Chief Financial Officer at Genentech, 1978-1984*, 2002

Diane Pennica, Ph.D., *t-PA and Other Research Contributions at Genentech*, 2003

Thomas J. Perkins, *Kleiner Perkins, Venture Capital, and the Chairmanship of Genentech*, *1976-1995,* 2002

G. Kirk Raab, *CEO at Genentech*, 1990-1995, 2003

George B. Rathmann, Ph.D., *Chairman, CEO, and President of Amgen, 1980–1988*, 2004

Regional Characteristics of Biotechnology in the United States: Perspectives of Three Industry Insiders (Hugh D'Andrade, David Holveck, and Edward Penhoet), 2001

Niels Reimers, *Stanford's Office of Technology Licensing and the Cohen/Boyer Cloning Patents*, 1998

Arthur D. Riggs, *City of Hope's Contribution to Early Genentech Research,* 2006

William J. Rutter, Ph.D., *The Department of Biochemistry and the Molecular Approach to Biomedicine at the University of California, San Francisco, volume I*, 1998

Richard Scheller, Ph.D., *Conducting Research in Academia, Directing Research at Genentech*, 2002

Robert A. Swanson, *Co-founder, CEO, and Chairman of Genentech, 1976-1996*, 2001

Axel Ullrich, Ph. D., *Molecular Biologist at UCSF and Genentech,* 2006

Daniel G. Yansura, *Senior Scientist at Genentech*, 2002

William Young, *Director of Manufacturing at Genentech*, 2006

Oral histories in process:

Brook Byers
Ronald Cape
Stanley N. Cohen
James Gower
William Green
Daniel E. Koshland, Jr.
Arthur Levinson
William J. Rutter, volume II
Mickey Urdea
Pablo Valenzuela
Keith R. Yamamoto

Interview History—Arthur D. Riggs

Arthur Riggs—"Art" to everyone who knows him—is a chemist-turned-molecular-biologist at City of Hope Medical Center in Duarte, California. He and his colleague Keiichi Itakura were interviewed for the biotechnology oral history series because of their seminal work in the mid-1970s on the two earliest research projects of Genentech, then a start-up genetic engineering company. Dr. Riggs provides in the oral history a full account of his life, but the emphasis is necessarily on his contributions to the contract research for Genentech on the hormones somatostatin and human insulin. He describes the earlier research "when the light bulb went on" for combining recombinant DNA and the chemical synthesis of DNA to build, clone, and express genetic elements. It was the application of these two techniques by Riggs and Itakura, in collaboration with Herbert Boyer and his laboratory at UCSF, that led to the successful cloning and expression in 1977 of the gene for somatostatin and the gene for human insulin the following year. The work was the proof-of-principle demonstration critical to Genentech's future in genetic engineering, and also demonstrated for the first time the utility of molecular techniques in the commercial sphere.

Three interviews were conducted with Dr. Riggs in his office at the Beckman Research Institute of the City of Hope, of which he is CEO and director. The institute's attorney, Gordon Goldsmith, attended the three interviews, commenting only very occasionally. His presence, highly unusual in oral history practice, was dictated by ongoing litigation between City of Hope and Genentech. It is the interviewer's impression, admittedly hard to substantiate, that the attorney's presence did not significantly alter the discussion except in the few instances (noted in the transcripts) in which he halted discussion. Dr. Riggs reviewed the transcripts, making very few changes and clarifications, and turned them over to Goldsmith and his successor, Greg Schetina. As he had with Itakura's transcripts, Mr. Schetina returned copies of the transcripts with no substantial additions or changes. By agreement with Genentech regarding the oral histories it supports, its legal department received transcripts of this and all other oral histories it supports to review solely for current legal issues. As in all instances to date, no changes were requested.

The Regional Oral History Office, a division of the Bancroft Library, was established in 1954 to record the lives of individuals who have contributed significantly to the history of California and the West.

Sally Smith Hughes, Ph.D.
Historian of Science
Program in Bioscience and Biotechnology Studies
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May 2006

Interview 1, January 12, 2005

[Begin Tape 1, Side A] ##[1]

Hughes: Well, there are three of us in Dr. Riggs' office today. The third person, who may not appear on the tapes is Mr. Gordon Goldsmith, who is the general counsel—

Goldsmith: Of the Beckman Research Institute of the City of Hope, Duarte, California.

Hughes: And we will proceed as though he were not in the room. I will start with my usual question, Dr. Riggs, which is, please tell me something about your background, going back as far as your grandparents.

Riggs: Ah, the grandparents. On my father's side, they came from Tennessee right at the turn of the century, so I think it really was 1900 or 1901. They came to a small town, Ceres, in California, near Modesto, California. They had a reasonably good-sized farm and were a farming family. I think they had eleven children that survived, so my father was one of the eleven that survived.

My mother's family and grandparents came at about the same time to the same city, and it was similar, I think. They also had about eight children that survived. So the grandparents lived and died in Ceres, and my mother was the next to the youngest, gosh, and born maybe about 19—ah, yes, she was actually born about the time they came to California, so she was born roughly at the turn of the century.

Hughes: Do you know why both sets of grandparents were attracted to California?

Riggs: Not really, no. On neither side do I really know why they came. Well, on my mother's side, they were actually part of an organized group of Methodists, I believe it was, so it was a planned community, planned by a—or at least the plan was influenced by the church connection. So it was a group that came out to this planned community in Ceres. My father's side, I have no idea why they came or how they came to be here.

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1. ## This symbol indicates that a tape segment has begun or ended.

Hughes: And had they been on both sides farmers in Tennessee?

Riggs: I don't really know. We haven't been able to trace my father's side back. My mother's side—yes, they were farmers in Ohio.

Hughes: And you were born when?

Riggs: 1939, I was born in Ceres, or I guess officially in Modesto, the hospital in Modesto. Lived there until about, I think I was six, when my parents—this was during the Depression—and they lost their farm. My father started working in shipbuilding at Long Beach Harbor. My mother was a public health nurse in Paris, California, which is near Riverside, near Temecula, between Riverside and Temecula. And then from age six on, I grew up in southern California. We moved to San Bernardino, so I started school in San Bernardino. So we must have moved to San Bernardino when I was six. I guess we were in Paris a couple of years before that.

Hughes: Do you have brothers and sisters?

Riggs: I have one sister.

Hughes: Younger or older?

Riggs: She's three years older.

Hughes: Tell me about family life.

Riggs: Well, [laughs] actually, it was—

Hughes: It's a broad question.

Riggs: Right. I went to public schools. My father did not have an education beyond the eighth grade. He started out as a farmer and had to stop school to help the rest of the family. Then he took up welding and pipe-fitting. I guess he was already middle age when he became a welder and pipe fitter. He was always working at something, so when he wasn't working as a pipe-fitter or welder, he was—most of the time when I was growing up, he was developing a mobile home park. So he managed to buy the land, and then he did everything, all of the electrical work, all the plumbing. So he just kept adding one space after another, and built up a fairly large mobile home park. All during this time, I was either helping him, or, what I think actually influenced me a lot, I would escape and say, "Dad, I've got to go study." [laughs]

Hughes: Which was a half truth?

Riggs: Which sometimes was true, sometimes was true. But I would tend to sneak away and read. I would read every chance I got.

Hughes: So it was more your attraction to the reading than trying to get away from working with your father?

Riggs: Well, it was a little bit of both. There were a lot of things to do, taking care of the grounds, killing gophers, digging ditches. And of course, when I was helping, that was the sort of thing that I was doing. Well, I just preferred to read. [laughs] I learned a lot. I learned the practical aspects of electricity, and also all the trade skills. One other interesting thing is I actually took a class in—what did they call it?—I think they called it mechanical drawing, when I was in junior high school. I wound up drawing the plans for the expansion of the trailer court, or the mobile home park. My drawings were actually used to submit the official plans to the city. I was very proud of that.

Hughes: Would you call your father entrepreneurial?

Riggs: Definitely. And the other thing I wanted to say: he was also an amateur pilot. He started early on, in 1910, when—let's see, probably more like 1920, when he was young. He started playing with airplanes not that long after the Wright brothers.

Hughes: My heavens.

Riggs: He flew and crashed various planes.

Hughes: Did he own a plane?

Riggs: He would build them, yes. I don't know too much about that because that was all done when he was younger. But he continued to fly, and I flew with him when I was in high school and junior high.

Hughes: Did you learn to fly yourself?

Riggs: Not really. I feel I could fly a plane if I had to, but I never got my license.

Hughes: Were you more interested in other things, such as the reading?

Riggs: I don't really know why I never wanted to become a pilot. Actually, I do remember. It was boring. [laughter] Yes, I'm serious. I learned how boring it is when I was a teenager. What I remember is, taking off is fun. But once you get up there, there's nothing to do. You sit there.

Hughes: It's like driving a car on a freeway, probably.

Riggs: Oh, much worse. Much more boring. Because once you're up about 15,000 feet or so, the ground is a long distance away. It doesn't change much, so everything is constant. And you sit there for hours doing nothing. So occasionally, he would

do something interesting, like put it into a controlled spiral, [laughter] take it into a stall—

Hughes: That you liked.

Riggs: I really loved that. I knew that a large part of it was not that exciting; there was no novelty factor.

Hughes: Do you think boredom, lack of novelty, is a theme in your life? I'm thinking ahead to science. Research, as you probably have experienced, has long periods of boredom when nothing much is happening.

Riggs: [laughs] That's true, but you can always escape to reading.

Hughes: [laughs] That tactic seems to reappear.

Riggs: Sure, there's a connection. I wanted to do my own thing. Maybe that was another reason, although I hadn't really thought about it. But it might be that since that was my father's thing, I wanted to do something different.

Hughes: Was he encouraging of your intellectual activities?

Riggs: Indirectly. Of course he was, but what I want to add to that is that it was more by example. So he taught by example. For example, about the time that I started college, so it was about 1960 and he would have been sixty years old, he decided to build an autogyro. Now, an autogyro is a helicopter where the forward motion is provided by a standard airplane engine and the blades are free-spinning. He went down to the local library, and he checked out books on helicopter engineering. I was going to school at Riverside at the time, so I was close enough, I came home. So when I came home, he'd ask me, "Hey, kid, how do you solve this equation?"

So I would show him how to solve the equation, which would be complex algebraic equations used for aerodynamic—helicopter engineering. I never had to show him more than once, and he understood the concepts of algebra, was able to, on his own, develop his own design for the blades, his own design for the hub. He would not copy anyone else's work or plans. He developed it entirely on his own, and built an autogyro that flew. Of course, I was amazed; I was in awe that it actually flew, and without doubt, I was influenced by that. No question about it.

Hughes: Did he patent it?

Riggs: No.

Hughes: It was always just a one-person autogyro?

Riggs: Well, he eventually built a two-person one, but it didn't fly very well. But he built several single-person planes or autogyros that flew very well. He was actually a test pilot. When he was seventy-five years old, he was still a test pilot. He started to make a few mistakes and retired from building autogyros when he was about eighty.

Hughes: And your mother?

Riggs: My mother was a nurse. She had a more traditional education, so she—

Hughes: She had more education than her husband?

Riggs: Oh, yes. She graduated from college and had a year or two of nursing training. So she got her R.N. and was a public health nurse all her life. While she was working, I had a lot of time by myself. You might say I was a latchkey child.

Hughes: Which is a little unusual in that era.

Riggs: A little bit, yes. But I loved it. [laughs] When I was in junior high, I was often alone for a few hours each day. Looking back, it was a great environment. I went to the public school and was always a pretty good student.

Hughes: Were you being pushed by one or both parents?

Riggs: Not really, in part, because they didn't have to. I was always interested in some subject and interested in reading, got interested in science early on. When I talked about escaping to read, well, I would be reading science fiction novels. So I was already interested in science.

Hughes: Had you narrowed—or when did you begin to narrow your interest to a specific science?

Riggs: Well, at some point, my parents got me a chemistry set, and I think that was probably when I was in the seventh grade. After I played with that and was just really enthusiastic about chemistry. I think I thought I was going to become a chemist early on. When I got into high school and took physics, I enjoyed that. So physics and geometry, algebra I really enjoyed. So nobody had to push me. I was self-motivated.

Hughes: Were there any teachers or adults outside the family that had a real influence?

Riggs: Yes. In junior high, there was a—his main job was the P.E. teacher, but he was also a pilot. He had an elective course; he taught a course in aeronautics. That was fascinating. I really enjoyed that, of course, partly because I had flown. But then I could learn some of the physics and navigation behind flying. So actually, I really enjoyed that and was favorably influenced.

Then in high school, it was a three-year high school—I forget the name of my teachers, unfortunately—but there were several teachers that did influence me. The one that I can remember, his name was a Mr. Gunderson, and he may actually have had the most influence of all. He taught a course in psychology. Actually, I did find it interesting at the time, even though I'd already decided that I was going to be a chemist or a physicist or something. He brought my attention to human behavior. He taught Freudian psychology, but—what do you say?—in a critical way. He didn't present it necessarily as being the absolute fact.

I remember deciding after taking the class—which was in large part a discussion class, so he tried to get the students involved in discussion--I remember thinking that Freud was wrong. [laughter]. Nevertheless, it had a strong influence on me because I paid attention to human psychology and how people interact. Iit was probably a good thing, because I probably needed to pay attention to that part of—

Hughes: But never with the idea that this was a career that you might pursue?

Riggs: No. That became important later on when you start managing groups of people. Most of the behaviors that I saw in others, and saw in myself, they weren't new to me. That's just exactly what Mr. Gunderson said. So I think it had a very strong influence and one that I think about every now and then.

Hughes: Well, before we move on to college, anything more to say about that early period?

Riggs: I forgot to say that I went to San Bernardino High School. Looking back, I got a very good training.

Hughes: Very unusual courses.

Riggs: Yes. I think I was lucky in that at that time; it was a very good school to go to.

Hughes: So you felt prepared when you got to college age.

Riggs: Yes. I went to the nearest good college, which was the University of California at Riverside , which had just been established a couple of years earlier.

Hughes: And you chose that because it was close to home?

Riggs: Really because it looked like it was going to be a very good school--small classes. The plan that they had in the beginning was for it to be part of the University of California, but more like a liberal arts college with a small number of students, a good faculty. So it was a university-quality faculty but with small classes. I said, "Oh, this is great." Oh, and it already had a science basis. It was the Citrus Experimental Station.

Hughes: And that was important to you?

Riggs: Well, I knew they already had a good chemistry and biology.

Hughes: Because you were pretty sure that was the way you were headed?

Riggs: Yes. I don't remember from junior high on ever thinking that I was not going to be a scientist. I didn't quite know what kind of a scientist I was going to be, but that was the only thing I was really interested in.

Hughes: Did you live at home?

Riggs: The first year, I lived at home. Rode my motorcycle the ten miles or so from my house to the college. But during the winter that became [laughs] uncomfortable. I decided that I really didn't want to commute on my motorcycle that far, so I stayed in the dormitory at the university for the next three years.

One thing I wanted to say in response to your question about whether I was well prepared: yes, I was extremely well prepared, and I didn't even bother to go to my lectures of the first year of physics class. I just went and took the exams. Didn't do that great; I think I got a B in the class. I was reasonably talented in physics, but mostly, it was just a repeat of what I'd had in high school.

Hughes: What about chemistry?

Riggs: Chemistry—I got a good start, and I was never able to quite do what I did for my physics class. [laughs] I did have to go to classes, and I did have to pay attention to the lectures. I've often wondered if my real talent wasn't physics. It was the second year in organic chemistry so I had to work hard in organic chemistry. But that was perhaps the first time that I was really the best in the class. I really liked it, and I just loved synthetic organic chemistry, where you start with some small molecule and you build up a larger molecule.

Hughes: Why did that intrigue you?

Riggs: I don't know. In part, I think it's a good fit for the way my mind works. In organic chemistry, you have to kind of visualize both the molecules and the process, and you say, "Here's where we are, and here's where we need to go." You have to develop a process in between. I was good at that, so I could do it.

Hughes: You mean, even down to the molecular level, how different groups are going to react? That was what you were visualizing?

Riggs: Yes. It's interesting; it's not just step by step. If you start here, you can't go step by step. You have to look towards where you want to go, and then out of the infinite variety of possible pathways, you have to pick the one that actually can work, using existing knowledge. It's a real challenge, and this is something that

synthetic organic chemists to this day still do. I think some people can do that better than others.

Hughes: It seems a very difficult task for you at that stage. I can imagine how you could visualize different pathways, but how would you have had the experience to know which one might be practical?

Riggs: Yes, it was—well, of course, we had to use what we were taught in the class. So there was some structure. But I think the point is that it was a type of thinking that I really enjoyed. After that class, I decided to become an organic chemist.

Hughes: Did you feel that you were unique in the class for being able to visualize in that fashion?

Riggs: Certainly not unique. There were other of my classmates that ultimately did become chemists who were quite good. But on the final exam, I think I did score the highest. At least, that's what I remember. [laughs] And the other thing I remember, someone asked me, "Art, how did you do that?" And I said, "I don't know; I was just guessing." I did a lot of guessing. He said, "Hmm, you're the best guesser that I know." There's something to that. Because there are so many ways to get—you had to select the right path.

[End Tape 1, Side A] ##
[Begin Tape 1, Side B]

Riggs: I think usually, we use different words for it. One word would be, take educated guesses. Or you play the odds. You don't do random moves; you make the move where you have the highest probability of success. So the risk-taking, being able to either consciously or subconsciously assess the risk involved, all that is probably not truly quantifiable, but I think some people are better than others.

Hughes: Well, you told me over lunch yesterday that you didn't feel that your abilities were particularly in the physical manipulation of science, but more in the conceptual.

Riggs: Yes.

Hughes: Do you think your experience in organic chemistry reinforced a conceptual approach to science?

Riggs: Well, I would still like to think that I can do experiments with my own hands. But I never really did become efficient at doing experiments with my own hands. I think partly it was, as a postdoc[toral fellow], I had a technician. I did do some work, and so I don't want to over-emphasize.

Hughes: I wasn't meaning to make it a complete dichotomy.

Riggs: Yes. I did spend my time in the cold room. It's just that I don't think I was particularly good. Maybe I was as good as the average technician, but probably no special skill in actually doing the experiments. It really helps to be able to focus and not make mistakes when you have to do 100 pipettings. You have to get all 100 of them right.Technicians that are 90 percent correct never finish any experiment correctly. [laughs] You can't be a 90 percent biochemist; you have to be almost 100 percent.

Hughes: Well, maybe that's where the boredom factor comes in that we were talking about earlier.

Riggs: Well, the ability to really focus in and stay focused, some people are definitely very good at it. To be able to even do the experiments while somebody's talking to them, I was never really very good at that. So I moved into more management and directing technicians and research associates.

Hughes: What more to say about Riverside?

Riggs: It was almost the ideal place for me. I think I would have been probably lost in a larger [institution] with huge classes, et cetera. I probably would never have gone to Caltech, for example. But at Riverside, I was able to get to know my professors. I worked for them during the summer, and that was probably the most important thing. So I actually did research during the summer, and they got to know me and I got to know them. Them getting to know me really helped when it came to being accepted at a first-rate graduate school, because they wrote letters—I never saw them—but they must have been very supportive, because I did get in at Caltech.

Hughes: Were you doing research that had significance for what you were going eventually to do?

Riggs: I actually had an interesting introduction to the realities of chemistry, dangers of chemistry. I was doing a reaction in a fume hood. Let's see, it had ether. The ether was overlaying an aqueous solution. I was shaking it, and it had a catalyst in it, and to everyone's surprise, the ether ignited. We had an explosion, which came out of the beaker, flaming ether all over me. I had safety glasses on. I remember looking down, and all the left part of my body was in flames. As I had been taught, I knew where the shower was, so I ran to the shower, which probably took maybe just a few seconds, and got it out. The amazing thing was that I was not burned at all. And it's because it was ether. Ether evaporates so rapidly that it burned sort of just off the surface. So I got no burns at all from the flames. There was some alkali in there, some sodium hydroxide, but that got washed off, so I didn't get any serious burns. A little redness from the alkali.Yes, in a way. I did actually do some original research in organic chemistry. I successfully made a compound using a different technique. I guess it had been made before, but I used a different technique to make a certain—what was it

called?—well, let's say a small organic molecule. Could have published it—should have published it, actually. But I got caught up in my graduate courses and I never provided my professor at Riverside with enough to get it published. So I never got it published.

Hughes: What a lucky outcome!

Riggs: Yes. Interestingly enough, I went right back to work. If anything, it made it more interesting. So if anything, I loved chemistry even more. [laughs]

Hughes: Well, tell me about the selection of Caltech. Did you consider other institutions?

Riggs: I'm sure I did, but I can only remember applying to Caltech. If I really did only apply to one, that was a pretty stupid thing to do. But it's moot, because I was accepted. Of course, Caltech had the reputation already, and so no question, once accepted, I was happy to go there.

Hughes: When had it become a given that you were going to go on to graduate school? Was it always in the books as soon as you knew that you wanted science?

Riggs: No, I really hadn't thought that far ahead, let's say, when I was in high school. Probably the second year in college when I took the organic chemistry class, I did decide to become a chemist. At that point, that's when I did decide to go on and did become a very serious student, started working quite hard.

One thing I forgot to mention: I also was interested in and influenced by my microbiology and biochemistry teachers at Riverside. I worked one summer for Dr. Carleton Bovell and Eugene Cota-Robles. I could have had a double major in chemistry and biology. I ultimately decided just to take the chemistry degree, so I graduated as a chemist, but I could have graduated with a double major.

Hughes: Why did you decide on just chemistry?

Riggs: I didn't; I took both.

Hughes: Yes, but you could have had a double degree.

Riggs: Yes, I could have, and I just had to write a thesis. But I had finished all the requirements for my chemistry degree, and there was no particular reason to get that double major. I was already accepted at Caltech.

Hughes: But it shows that you were leaning—

Riggs: I was interested in biology. The plan was to apply chemistry to biology. That definitely was the plan from my college days.

Hughes: And how did that idea occur?

Riggs: I ought to describe that I became interested in biology in part because I liked it. [laughs] My mother was a nurse, and so I think I should give her credit that I was aware of biology and medicine. My simple-minded plan was since I was good at chemistry, I was going to be a chemist that applied my skills in chemistry to problems in biology and medicine. I think it's logical.

Hughes: Did you go so far as to say, "I am not so interested in just the theoretical aspects of this, but I want to do science that results in practical applications?"

Riggs: I think I was always interested in practical applications, yes. Yes, thinking back, yes. I intended to solve problems, including medical problems, even back when I was an undergraduate.

Hughes: Perhaps your parents had some influence on that approach?

Riggs: Probably.

Hughes: I mean, they were both doing practical things.

Riggs: I'm sure they did.[laughs]

Hughes: All right, so you arrived at Caltech [1961-1966]. Now, this was a very different place from Riverside, was it not?

Riggs: Well, interestingly enough, not that different. I do feel I've lived a charmed life, and one was to go to Riverside when it was still a very small school; I really got excellent training. I came to Caltech feeling that I was of course—what do you say—the country hick going to the best place for molecular biology in the world. I was actually surprised that I was very well prepared in many ways. My chemistry training was excellent. I did take classes in mathematics and my math was fine; didn't have any trouble with physics, and did pretty well in the classes. We did have to take classes the first year. Sort of to my surprise, because I was a bit intimidated. Sort of to my surprise, I did pretty well.

Hughes: Did you have any famous teachers in that first year?

Riggs: Yes, definitely. And also I wound up doing my research right next to Ed[ward B.] Lewis. Ed Lewis about twenty years later got a Nobel Prize for the work he was doing when I was there at Caltech. I got to know Ed Lewis quite well, in part because I was working on *Drosophila*, doing a little bit of—well, it was *Drosophila* biochemistry really. So Ed Lewis might be the only one—well, I'm drawing a blank on the most well known person there.

Hughes: In *Drosophila* research?

Riggs: No, it's not the area.

Hughes: In the second year, then, you began your own research?

Riggs: Yes.

Hughes: And what was that, and in which lab?

Riggs: Well, I settled on the laboratory of Dr. H. K. Mitchell, [Hershel] Mitchell. He was a developmental biochemist using *Drosophila* as the model system. So for the first couple of years, I ground up *Drosophila* and studied some—I forget now actually what the project was. After about a year, I decided that no one would ever get anywhere grinding up whole animals, which was basically what you were doing, and then trying to do biochemistry on a whole animal. I think there's 300 different tissue types.[laughter]

Hughes: That was the standard for the lab? Is that what most people were doing?

Riggs: Well, at that time, you couldn't do anything else. So what else could you do? You could do genetics. My boss, Mitchell—everybody called him Mitch—so Mitch did do some genetics, and so he used genetics to help the biochemistry. But I was more of a chemist, and I wanted to study the chemistry that was the basis of genetics. And I did. I decided that I was never going to get anywhere using *Drosophila*. And at the time, I was correct. So I decided to leave the field of *Drosophila* and started—[knock at door, tape interruption] I decided that no biochemist could get very far studying *Drosophila*, so I started looking for simpler organisms. Maybe I was influenced by Max Delbruck, because he was also at Caltech, and even though I didn't get to know him very well, nevertheless, I was influenced by him. So I started looking around for the simplest possible free-living organism, and decided to study mycoplasma, which was totally different from anything that Dr. Mitchell had done. So I went to him and I said, "I'd like to do this." His policy was to let his students do anything they wanted.

Hughes: You were lucky there.

Riggs: Yes, I was, right. In some ways, that's good, but it also is a sink-or-swim situation. Students really don't have that good a perspective—I didn't, anyway, and I think it's generally true. So I really didn't know quite what I was doing. It turned out all right. But I wound up doing a thesis on DNA replication, which was totally different, and I didn't really plan to go that way. But I started working on this mycoplasma study, and I developed a technique—I shouldn't say developed—I took a recently published technique and got it operational. Then I collaborated with another graduate student. [interruption, pause]

So I started collaborating with a fellow graduate student, Joel Huberman, and that resulted in—you might say—a breakthrough result which led to a really dramatic increase in our understanding of DNA replication in mammalian cells.

So the Huberman and Riggs study became a citation classic, and it worked out extremely well.

One reason I want to mention it is that when Joel Huberman and I decided to do the experiment, which involved autoradiography—so it was an autoradiography DNA replication experiment—I went to my mentor, Dr. Mitchell, and he said, "No, don't do that. You need to finish up your thesis." And then Joel Huberman went to his mentor and said, "We'd like to do this experiment," and he also said—and this was Giuseppi Atardi—"No, don't do that experiment; you need to do the experiment you're currently working on."

So we snuck in at night, we literally did. We didn't sneak in, but we came in at night and did the experiment anyway. And then after we got results, which were rather dramatic, fortunately—the first experiment gave very interesting results—then we went back to our professors and said, "Can we continue on these experiments?" [laughs] And they said yes.

The last point I want to make is that they also did something that most professors or scientists can't do any more, and that is, both Mitchell and Atardi said, "Since you did the work on your own, against our advice, we will let you publish it alone. So we don't need to put our names on the paper." We were fine with it; we liked them; we had good relationships with our professors. So we asked them to be on the papers, but they declined. [looking through papers] As I said, that paper became a classic, and it certainly launched both our careers in science.

Hughes: Is that the 1968 one?

Riggs: Yes.

Hughes: Well, let's go back. You had been working with *Drosophila* and realized that it wasn't the organism for you. The phage group man that you mentioned—

Riggs: Max Delbruck. He was working with a bacteriophage, a virus that infects *E. coli.*

Hughes: Where does mycoplasma come in? How did you have that idea that itmight be a good organism to work with?

Riggs: Mycoplasma? Oh, gosh. I was looking through the literature—I had read a paper where somebody claimed that they had a mycoplasma that had a very small genome, and so I started studying it.

Hughes: I'm struggling to remember mycoplasma; they're not strictly a bacterium, are they?

Riggs: Well, they're related in that they're like bacteria but they don't have a cell wall.

Hughes: You chose it because of its small genome?

Riggs: Yes.

Hughes: Why wouldn't you have used the same organism that the phage group was using?

Riggs: Well, one answer is I don't know, I don't remember. [laughs] But the other answer is that there was already somebody working on that, and so I was trying to do something a little different.

Hughes: You wanted to have your own ground. Huberman—what was his background?

Riggs: He was studying chromosomes and preparing chromosomes from mammalian cells.

Hughes: I see. Was he the one who introduced the idea that maybe DNA was the thing to be working on?

Riggs: Oh, no—I was working on this genome size of the mycoplasma. I was working on the DNA and the way of spreading out the DNA so you could see it in the electron microscope. I also did the autoradiography where you stretch the DNA out and you can actually see it in a light microscope because it's tritium labeled. So he had the expertise for growing mammalian cells; I had the expertise for doing the techniques for visualizing the replicating DNA molecules.

Hughes: When you worked with *Drosophila*, had you been focused on DNA?

Riggs: No.

Hughes: So that was quite a leap.

Riggs: I developed the autoradiography and electron microscopy on my own. Well, not on my own—the decision to do it was mine. Then I got help from everybody at Caltech, of course.

Hughes: I know that DNA was very much in the wind. What was this, maybe a decade or so after Watson and Crick [1953], but it was not a given at that time that a biochemist was going to be interested in DNA, right? Why did you get into DNA biochemistry?

Riggs: The question I was asking was: What is the true size of the genome of the mycoplasma? So then I searched the literature for techniques that allowed me to determine that.

Hughes: Why would that even occur to you?

Riggs: Why was I doing that?

Hughes: Yes. Because you hadn't been doing that.

Riggs: That's right, that is correct. I was looking for a project to do that I thought would be interesting and would contribute. As I mentioned earlier, I think I was influenced by the Delbruck influence in that I was looking for the simplest system—the simplest autonomous organism that had a cell membrane and was able to grow and divide on its own. So I was intrigued by that. As I think about it, I think Wally [Walter] Gilbert may have published a paper that I saw. I think I'd want to check that, but I think that he was the author or one of the authors of a paper that I saw that did influence my graduate work.

Hughes: We're talking about mid-sixties?

Riggs: That would be about 1963 that I started that work.

Hughes: Had you been following all along the work on DNA and RNA?

[End Tape 1, Side B] ##
[Begin Tape 2, Side A]

Riggs: There was a handful of centers that were at the forefront of what's now called molecular biology. At that time, it would have been DNA-RNA-protein work.

When I started my career, the DNA structure had been accepted. The question they were working on was, what was the genetic code? So there was a lot of work being done at Caltech and elsewhere on genetic code. And then part of that is, how are proteins made? While I was a graduate student at Caltech, a person at Caltech whose name I forget right now, and others, like [Francois] Jacob and [Jacques] Monod, for example, started getting evidence for what's now called messenger RNA. So that was unfolding, becoming understood, all during this time. So I was at the right place for all of this work.

Hughes: You were going to seminars and talking to people and up-to-date on the latest?

Riggs: Yes, sure. I was at one of the handful of centers for molecular biology at that time.

Hughes: You said there were dramatic results from the very first experiment that you and Huberman did. What exactly were you doing, and what were the results?

Riggs: [laughs] Well, as always, the details of these experiments are a little hard to describe, but I'll try. DNA is composed of the four nucleotides, one of which is thymidine. At that time, you could obtain tritiated thymidine, so you'd get radioactive-labeled thymidine. If you feed radioactive thymidine to cells, it's taken up and incorporated into the DNA. So the DNA will become labeled with radioactivity, and it will become labeled first where the DNA is being replicated.

At the time, no one knew anything about how mammalian DNA was replicated. We gave the cells a pulse, we added radioactive thymidine, and then we immediately very gently lysed the cells. In essence, you lyse them in a detergent, and then you let gravity drain from a chamber, the lysis chamber. The DNA molecules get hung up on the surface of the chamber.

So then we were able to overlay these DNA molecules, some of which were labeled with radioactivity. We were now able to overlay that with photographic film, and so we did that. Then you put the slides—this all was done on a slide—in the freezer, and we let the radioactivity expose the film for about four months. That's actually what we did. So we came in, and the experiment didn't take that long, really, just a few nights. Then we put it in the freezer and waited four months. When we took out the slides, developed the film, we could actually see the replicating molecules. This had not been done before for mammalian cells. It gave a lot of information.

Hughes: Did the paper cause quite a stir?

Riggs: Oh, yes. And to this day, it's still cited quite often. Yes, it was well received.

Hughes: That was your formal introduction into DNA molecular biology, wasn't it?

Riggs: That's right.

Hughes: Well, what then happened?

Riggs: So then I went to the Salk Institute [1966-1969]. I might as well say that I had another idea as a graduate student. It's hard to describe—I'll simplify. I thought I could use the substrate [pause]—I have to start over.

It had just been figured out how genes were turned on in bacteria. Jacob and Monod learned that there was a derivative of a sugar, a derivative of galactose, that would cause the gene for beta-galactosidase to turn on, and the sugar that caused *E. coli* to turn on beta-galactosidase had just been learned. I said, "Hmm, we could take that—" It's called an inducer. So I could take that inducer and I could attach it to a solid material and make a chromatography column. Then this should pull out the lac repressor.

My wife's parents lived in San Diego, and I knew the Salk Institute was a good place to go, and I knew that at the Salk Institute, there was a person named Melvin Cohn, who was working on the lac repressor, or had done work in France on the lac operon—before he came to the United States.

Hughes: He'd been in the Jacob-Monod group, hadn't he?

Riggs: Yes, he had been, and then he went to the Salk Institute. Well, he went to Stanford, and then to the Salk Institute.

So while I was down there one weekend visiting my in-laws, I stopped by and told this great idea to Melvin Cohn at the Salk Institute. He said, "You know, Art, that is a good idea," and he really did say something like that. He said, "That is a really good idea. I had that same idea myself ten years ago," [laughter] "and I tried it, and it didn't work." But then he went on to say, "But we've learned a lot since then, and it is time to try again." So he was actually very supportive. He said, "Yes, that's a good project; I'd be happy to have you in my lab."

So I went to the Salk Institute [1966] and started work on the lac repressor, trying to use what later became—it was called affinity chromatography. So I tried to use affinity chromatography to isolate the lac repressor, and that didn't quite—with hindsight, I think it would have worked, but we got scooped by Wally Gilbert and Benno Muller-Hill. They isolated the lac repressor—or at least identified it in extracts at about the time I was starting my project on using this other approach to isolate the same repressor. So my project switched to purifying the lac repressor. Gilbert and Benno Muller-Hill had identified it in extracts, but they had not purified it. So I did start working on the lac repressor and the lac operon. And of course, this is relevant for the somatostatin and insulin work.

Hughes: Yes, of course it is.

Riggs: This is when I really started, you might say, working on that project. I started working on the lac repressor and trying to understand how the lac promoter worked. So that was my project as a postdoc.Well, that work went quite well, and I actually was the first person to have pure regulatory proteins in a test tube. I published several papers on the lac repressor, which were very well received. So I was well known as one of the leading researchers on the lac repressor and the lac operon.

Hughes: A very hot field at the time.

Riggs: It was, yes.

Hughes: How much interaction did you have with Melvin Cohn?

Riggs: Well, he taught me a lot. The other thing I wanted to mention is that he had already switched, so his primary interest was no longer on gene regulation in bacteria. Almost his entire lab had been converted to immunology. There was a lot of experimental work going on, but he himself had become, you might say, a theoretical immunologist, trying to understand how the immune system can distinguish self from foreign. The question of how we can recognize self was his primary interest. His laboratory was almost entirely devoted to immunology. It was a large lab, and virtually all aspects of immunology was represented there in his laboratory. That's relevant also for later work that I did.

Melvin Cohn's wife, Suzanne Bourgeois, and I—and then later one other person joined us—so there were three of us that were working on the lac repressor. All the other people in Melvin Cohn's laboratory were doing immunology. Of course, all the lab seminars were mostly on immunology. I actually became a pretty good immunologist also during my stay in Mel Cohn's laboratory. He was a very dynamic person, and he was very influential. In particular, he liked ideas, and he liked to—well, basically, to think and debate and discuss how things work. He was very good. I was really fortunate to be there.

Hughes: Despite his new interest in immunology, was he engaged in what you were doing?

Riggs: Of course, of course. I actually got to see a lot of him, in part because I was working with his wife. [laughs] So yes, he kept very close contact. Yes, he'd come by once a day probably and say,
"What's going on?"

Hughes: Were there any notable differences in the cultures of the two places, the Salk Institute and Caltech? Were there similar interactions and scientific stimulation and all of that?

Riggs: Yes, I'd have to say it was very similar. Both, of course, were I'd say at the forefront of molecular biology research at the time, and they were both—yes, very similar.

Hughes: How were you thinking of yourself at that point?

Riggs: By then, I was a molecular biologist. By then, the term had become common. I got my degree in biochemistry, but that was just because molecular biology terminology hadn't been invented yet. But really, I was doing molecular biology. It's genes, what they are, how they function. I was studying that, both as a graduate student and as a postdoc.

Hughes: Anything more on education?

Riggs: I think that pretty well covers it.

Hughes: Looking at your CV [curriculum vitae], you went immediately to City of Hope [1969]. Why City of Hope, and did you consider anyplace else?

Riggs: I didn't. I did not consider anyplace else. The reason I came to City of Hope was in large part because Melvin Cohn—whom I've already mentioned—you might say had a theoretical bent or he liked theory. He said, "At the City of Hope, there's this genius called Susumu Ohno. He's a very interesting person." One of the things he was working on was X-chromosome inactivation. X-chromosome inactivation, as I learned first by reading Ohno's papers, is a phenomenon that occurs only in mammals, and it's a phenomenon that's very difficult to explain at

the molecular level. The cells of female mammals start out with two X chromosomes. If you use inbred mice, these two X chromosomes have identical DNA sequences. So you start out with two completely identical large structures called chromosomes, and we now know the genes on both are active, but we didn't know at the time. And then, at about the time the mammalian embryo implants into the uterine wall, one of the X chromosomes gets totally silenced. That is, all of the genes on one X chromosome get shut off. The other one stays active. So all cells in a female mammal have only one active X chromosome. So they're just like the males in that sense. The males have only one active X chromosome. So the difference is that the female cells have this inactive X chromosome still there, so every nucleus in every cell has this second chromosome, but it's totally shut off and totally functionally silent.

I read that, and I said, "Wait a second. How can you explain two identical DNA sequences being treated so differently?" I forgot to mention that once the initial decision is made, the inactive X chromosome is inherited somatically—all progeny cells retain the same X inactive. You get one X from the paternal parent and one X from the maternal parent. If in a given cell, the paternal X is the one that was silenced, all of the progeny of that cell will have the paternal X silenced. So they will remember the state of differentiation or the inactive state of the X chromosome. How can that be? And so that puzzle just attracted my attention, and it still does. [laughs] I am still working on that. That's the reason I am at the City of Hope. That's the simplest answer.

I came and I talked to Sosumu Ohno, who had just become director of the biology department here in this building. So he was downstairs in this building. Like Melvin Cohn said, he was really a brilliant person. So I decided to come here for that reason—to work on X-chromosome inactivation.

Hughes: Was that the sole focus of his laboratory?

Riggs: No. By the time I got here, [laughs] he had pretty much lost interest in X-chromosome inactivation and had moved on to other things. Which I don't think we need to go into. He was interested in immunology, and he did maintain that interest until his death a couple of years ago.

Hughes: The reason that you were basically interested in the X-chromosome inactivation was because it had to be a question of gene regulation?

Riggs: It was gene regulation—

Hughes: So it was a continuation of the work you'd been doing at Salk, in a certain sense.

Riggs: That is correct. A continuation, and it was a mammalian phenomenon. I was needing to obtain funding. I got here in 1969, when I'd say the golden age for funding was just ending. It was beginning to become hard to get grants. I was wrong, but I thought that it would be easier to get grants if I was studying a

mammalian gene regulation phenomenon. And I think that was true. The other funding continued, so most studies in the lac repressor were also funded. I didn't abandon the work on the lac repressor. Actually I continued working on the lac repressor when I established my own laboratory here.Oh, by the way, I didn't come to work for Dr. Ohno. I came to work in his department because I knew I would have an environment that was interesting.

I forgot to mention one other person who was quite influential, and that was Dr. Charles Todd, who was an immunologist and studying the genetics of immunology. His laboratories were on the west side of this building. So I came here to be near and so I could interact with Dr. Ohno; I also came so I could be near and interact with Dr. Charles Todd. So I had both my interests, immunology and gene regulation and X-chromosome inactivation as gene regulation. I thought this was kind of the future, so that's where I was going. Then what I had going was the lac repressor work, and I continued doing that. That's what I initially got funding for from NIH [National Institutes of Health]. I was successful in getting grants, and so my work took off pretty well.

Hughes: Were you still in a minority in terms of working with mammalian gene regulation? I think of the earlier work as being focused on much simpler organisms.

Riggs: Well, yes and no. I consider myself a molecular biologist. I'm not a cell biologist or anything. Many and probably most of the molecular biologists were studying *E. coli,* or yeast, or *Drosophila*. I wasn't alone. There was a large number of developmental biologists that were interested in mammalian gene regulation. Most of them probably were not card-carrying molecular biologists, but they were rapidly becoming so. It was already a reasonably large field. Only a small number were studying X-chromosome inactivation, so I liked that. It was a nice niche. I did see a niche that had a fascinating problem, and I correctly thought it was a niche that I might be able to both contribute something to and get funding for.

Hughes: Your point is that you came as an independent researcher? You were not working under Ohno.

Riggs: Right.

Hughes: How did you negotiate that? You were just a wet-behind-the-ears Ph.D.

Riggs: They were recruiting for an independent investigator.

Hughes: So it wasn't anything you had to specifically negotiate.

Riggs: No. They were recruiting for that, and I was actually not their first choice, but their first choice turned them down, and so— To make it simple, I'll leave it that way. [laughs]

Hughes: So that was 1969. What happened after you arrived?

Riggs: Well, first of all, things went pretty well. I did find this a good place to do research, and I continued to work on the lac repressor. Most of the work in my laboratory was on the lac repressor until, let's say, 1975, in part because it took me almost that long to have any really good practical ideas about X-chromosome inactivation. We'll come back to that, but let me stay more focused on the lac repressor, which led to the somatostatin and insulin projects. So I was working on and had a reputation for being one of the experts on the lac repressor. Around 1973, I think it was, maybe '74—roughly that period—a postdoc from Caltech—his name was John Rosenberg—came to my laboratory. He was a postdoc in Richard Dickerson's laboratory at Caltech. Richard Dickerson and John Rosenberg were x-ray crystallographers. Their goal was to obtain protein crystals and solve the structure of the crystal, and get really detailed information about the proteins.

So John Rosenberg came over, and we chatted for a while. He said, "As my postdoc project, I would like to crystallize the lac repressor and determine its structure." I said, "Well, that's a great idea, but several others have been trying to do that for four or five years, and they've not been successful. So I think that you should not do that as your postdoctoral project." So he went away, discouraged. I don't remember exactly how long it was after that, but let's say a few months later, Dr. Ohno gave me a letter and said—

[End Tape 2, Side A] ##
[Begin Tape 2, Side B]

Riggs: —"Would you be interested in this?" I looked at it, and it was a letter from an organic nucleotide chemist by the name of Keiichi Itakura, [laughs] and he said, "I'm looking for a job." As I recall, the last sentence was, "And I've just completed the synthesis of the lac operator." Now, the lac operator is the DNA element to which the lac repressor binds. So I immediately said, "Yes, this is interesting." Then the next thing I did was call up John Rosenberg at Caltech, the postdoc that I had been talking to. I said, "We've got an interesting possibility here to do what no one has attempted, because they haven't been able to get the material to crystallize the lac repressor bound to its natural target, the lac operator." I knew, and I think it was common knowledge, that the substrate for protein often stabilized the protein. Basically, it made it an entirely new ballgame as far as crystallization goes. I said, "So I think we ought to rethink this." So then that started the ball going. The answer was yes, and of course, he got very excited about it, and I was excited about it. So we started talking with Ohno and Richard Dickerson, and we said, "Hmm, it would be great to get Dr. Itakura here, because this would open up a number of possibilities." So we invited him out, and right from the beginning there was the four of us that were interested.

Hughes: And he was coming from Canada?

Riggs: He was coming from Canada, from Dr. [Saran] Narang's laboratory. So we invited him to come out, and Dr. Itakura gave this seminar which was quite good, and I decided, and I think really everybody decided, that we definitely wanted him to come. So we decided to try to get him. To make a long story short, he came. I guess the way to think about it is that Ohno was willing to provide salary for Itakura, but we didn't have any space at all. Dickerson didn't have the resources for a salary, but he had space. So we offered Itakura a tenure-track job here at the City of Hope, but we had no space. So he wound up working in Richard Dickerson's laboratory for a while, and that's where he started making DNA. The plan was that as soon as we got space and laboratories for him here, he would come back to the City of Hope. Now, I don't mind saying, because I think Caltech made a wrong decision. After Itakura had been there a while, he realized that it would be a good thing for him to stay at Caltech if he could. Well, they said no.

Hughes: Do you know why that was?

Riggs: No, I don't. Except that it's extremely hard to get a position at Caltech. And he had a good thing going here. So we wound up providing a faculty-level position for Itakura here at the City of Hope.

Hughes: Do you remember when exactly he came?

Riggs: Oh, this would have been about 1974, roughly. Plus or minus a year.

Hughes: He was at Caltech in '74-'75—

Riggs: There we go.

Hughes: —and became an associate research scientist here some time in 1975.

Riggs: Yes.

Hughes: I'm interested in this Caltech group because they figure in some of the research that's coming up. How does Richard Scheller fit into this story? He was a postdoc—or was he a graduate student?

Riggs: He was a graduate student at Caltech, and he started working for Richard Dickerson, but then really for Itakura. So when Itakura was at Caltech, Richard Scheller started learning organic DNA synthesis. Then Itakura came over here [to the City of Hope], and Richard Scheller continued to be a part of the team, so to speak. But at some point, I think it was roughly a year after he started learning organic chemistry and especially the nucleotide chemistry that Itakura was doing, he decided that really was not the research for him. So he decided to drop out of the project, and he went to work for someone else, whose name I forget.

Hughes: At Caltech?

Riggs: Yes. So he completely switched. He remained a friend. We liked him, and he liked us, I think. And he has had a great career doing other things.

Hughes: But before he did other things, there was that business about the linkers.

Riggs: Yes, he was involved in the linkers.

Hughes: That was before he switched, and he got into the neurobiology.

Riggs: That's correct. As a student, he was working for Itakura, I think for about a year.

Hughes: Leaping ahead, I remember that one of the contracts that Genentech had was with Caltech.

Riggs: Caltech was included; yes, it was. That was at the very beginning. Scheller was involved.

Hughes: It was because of Scheller, was it not, that the contract was set up?

Riggs: Yes. Scheller had learned how to do nucleotide synthesis. I think he was actually going to come over here—but he would remain an official graduate student at Caltech—and he was going to make the DNA for somatostatin. That was part of the original thing. He actually was even involved, I think, in making some of the first fragments, which were not successful. He actually made some of the DNA that I played with in the summer of '76 when I was up in Boyer's lab.

Hughes: There must have been a DNA synthesis lab that Itakura had set up at Caltech?

Riggs: You know, I don't remember where it was. Yes, it could have been at Caltech; I don't remember.

Hughes: Because Scheller must have been working under the aegis of Dr. Itakura, right?

Riggs: Oh, absolutely. So what I'm not remembering is exactly when Itakura's labs were completed here, and he moved over here full-time.

Hughes: I don't have the month, but it was 1975.

Riggs: Yes. I also don't remember where Scheller was actually working. He could have been working here; he could have been working there. I don't know.

Hughes: My memory is that he was working always at Caltech. But I know that he originally was supposed to be part of the somatostatin project.

Riggs: Absolutely.

Hughes: And of course that's the basis for that later great story, that this graduate student became a paper millionaire when Genentech went public, remember? Because he had been given shares of Genentech's stock.

Riggs: That's right. And I think it is a great story. But he did make a decision to disengage—

Hughes: To move on, yes.

Riggs: —from that, and his career went a totally different path. I think it's just wonderful that he wound up being the research director for Genentech.

Hughes: Okay, so Itakura comes to City of Hope, and there's no DNA synthesis going on here before he arrives, is there?

Riggs: Before he arrived, no, that's correct.

Hughes: So what about setting up a lab, or is that a better question to ask of him?

Riggs: Well, it's probably better to ask of him, because my role there was just to basically convince the institution that they should spend the money to get him started, and they did actually. There was some seed money that came from the City of Hope to get him started. So I helped get the City of Hope to make space decisions and get his labs going. As far as the details of planning the labs, whatever, he did that.

Hughes: I'll ask him.[2] Were you collaborating from the start?

Riggs: Yes.

Hughes: What was the first project?

Riggs: The first project was to crystallize the repressor-operator. I've called that project the great failure because we never succeeded in getting good crystals of the repressor-operator complex. But it led to a number of really incredible things. The Genentech story is not the only important result of the project. Rosenberg eventually did get crystals of a restriction enzyme. I think it was Eco R1, if I remember right. So the first crystal structure of restriction enzymes came as a spin-off. Rosenberg started working on the lac repressor; that never worked. But because he got interested in restriction enzymes, he wound up crystallizing Eco R1.

Hughes: Do you remember what year that was?

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2. There are oral histories in this series with Itakura, Scheller, and others involved with early Genentech research.

Riggs: It was mid-seventies, maybe.

Hughes: Was Eco R1 by then available commercially?

Riggs: I don't think so.

Hughes: So did the enzyme probably come from the Boyer lab?

Riggs: Yes, I think Pat [Patricia] Greene was preparing it, and I think she and John Rosenberg collaborated. I wasn't part of that, but I think that's the way that worked out.

Hughes: Is the next step in this story your two visits to the Boyer lab?

Riggs: Yes, I'll start with my first visit. I had gotten to know Herb Boyer in the early seventies. I don't know exactly when. I'm pretty sure we met at a Gordon conference. I had the lac operon DNA already in my laboratory here at the City of Hope. Several people had it; I wasn't the only one, and I didn't do the cloning myself. But I had the lac operon, I had a lambda plac phage that had the lac operon in it. Boyer was interested in cloning beta-galactosidase, and so we got to talking about it, and we agreed to work on it. So I went to his laboratory, I think it was around '73, took the lac—

Hughes: Had the first cloning paper come out?

Riggs: Maybe not.

Hughes: The first paper on recombinant DNA by Boyer and Cohen came out in November '73.

Riggs: Plus or minus a year here, but before it [recombinant DNA] was common knowledge, and I think probably before publication, Boyer came here and gave a seminar. So at that point, I knew that the Eco R1 could be used for sticky-ended cloning purposes. I think it was part of that—so it would actually be logical or sensible, but I don't remember precisely. I did go to Boyer's laboratory with the idea of one of his people cloning the lac operon and most of the beta galactosidase gene. So we did some work. I stayed there for a short time, and we actually tried to get some experiments done. I don't think they were particularly successful.

Hughes: No publication came out of that?

Riggs: I don't think so; let's see. [looks through his bibliography] [Robert] Helling—maybe they did.

Hughes: Helling was there in 1973.

Riggs: I think Boyer and maybe Helling did publish something on the electrophoresis of fragments from the lac operon.

Hughes: Yes, I don't see anything that you did together until the paper on synthetic lac operator cloning.

Riggs: Yes. But there were a couple of experiments there. Then basically I left the plasmid there with him, so that was when he first started working with the lac operon.

Hughes: Now, did you know recombinant DNA technology at that point?

Riggs: Only because I had learned from Boyer.

Hughes: At that point, on that first visit?

Riggs: I think, yes. He came here and gave a seminar very early on, and that probably was a few months before I went there for that summer.

Hughes: And we're talking about 1973?

Riggs: Yes.

Hughes: Do you want to say something about your impressions of his lab?

Riggs: Well, I certainly enjoyed my visit there. [laughter]

Hughes: Do you remember who was there on that first visit—was Helling there?

Riggs: You know, I don't remember. The person that comes to mind is Mary Betlach, but I can't really tell whether it was during that visit or later on. The truth is, I don't remember.

Hughes: Herb Heyneker wouldn't have been there yet, because he didn't come until 1975.

Riggs: That's correct; he was not there.

Hughes: Mary Betlach was maybe teaching you recombinant DNA technology?

Riggs: It's possible, but I don't remember. That's relevant mainly in that Herb Boyer and I were already colleagues and friends.

Hughes: You mentioned being at a Gordon conference, and that that might be where you first met Herb. Were you at the Gordon conference in June '73 when he talked spontaneously about his work [with Stan Cohen], and that tripped off the whole recombinant DNA controversy? Dieter Soll? [and Maxine Singer]—the names

are not coming to me today, I'm sorry. But anyway, they wrote a letter that started the ball rolling that formed the Berg committee that led to Asilomar that led to the NIH guidelines.

Riggs: You know, I hadn't really thought about it. I could easily have been at that Gordon conference.

Hughes: Boyer gave an add-on talk, just because there was concern. That was when the question of biohazards arising from recombinant research first arose.

Riggs: I don't remember. But I was going to the Gordon conferences, and it is definitely possible that he talked about his work there. And that may have been why I invited him here, because we did invite him here—and it was early on. I think it was before publication [of the 1973 paper on recombinant DNA], so all that would make sense. This sort of set the stage for the later work. I was pretty well up on what was going on in Herb Boyer's laboratory; I was in communication with him.

The next step which is relevant to the somatostatin project, and it really brought Heyneker into the picture and solidified the relationship with the Boyer laboratory, was started by sort of a failure, or, let's say, a lack of meeting expectations, that's probably a better way to say it [laughter]. Itakura was a bit over-optimistic about the amount and quality of DNA that he could make. So he came and worked I think for about a year roughly—he worked hard, day and night. He was probably working alone at this time. He took a long time, did a lot of work, and he made some lac operator DNA. Some of it was functional, and I did work in my laboratory to check it out, and we published some papers on—

Hughes: How did you prove that it was functional?

Riggs: I had the lac repressor, and I had developed a filter-binding assay, which we don't need to get into. So I had developed methods from my previous work to study protein-DNA interaction. So I applied those same methods to checking out the interaction of the repressor to the material that Itakura synthesized. We proved that what he made did have functional lac operator in it. It would bind to the repressor, but we suspected that the purity was not good enough. The amount was definitely not enough for crystallization. So the crystallography project really was dead in the water, at least at that point.

Actually, I don't mind giving credit to or pointing out that Wally Gilbert came to Caltech, gave a seminar there, and he talked about the use of DNA ligase for blunt-end cloning. So we learned from Wally Gilbert for the first time about blunt-end cloning. That was just at about the time that we were learning that the material that Itakura made was not quite good enough. You know, decent, but not— So we had a meeting with Wally Gilbert, Richard Dickerson, John Rosenberg, Itakura, and myself. We were sitting around, and during that

meeting, the light bulb went on, and I said, “Hmm, Wally, if we made double-stranded DNA with a restriction enzyme site in it, could we use your enzyme to blunt-end ligate or join it to, for example, the lac operator?” Wally thought about it for a second and he said, “Yes.”

I knew that Herb Boyer had had a chemist in Germany, I believe it was. He had had him make some Eco R1 DNA, so he had some DNA that had the Eco R1 site in it. I called up Herb Boyer and I said, “Herb, I would like to collaborate with you. I’d like to blunt-end ligate the Eco R1 site that you have onto the lac operator that Itakura has just made so that we could clone it.” I didn’t patent it, and nobody ever did. But at that time we became aware that the best purification method possible was cloning. So it didn’t matter if Itakura’s DNA preparation was not perfect; as long as a few molecules were, you could pull them out.

Hughes: Was that the first time that you saw the juxtaposition of DNA synthesis and recombinant DNA? Or had that always been apparent to you?

Riggs: I would say that’s when it really became the way to go. [laughs] Prior to that, we were focusing more that Itakura would make enough DNA, and then we would then mix it with lac repressor, get crystals. So we were not really thinking about cloning. It was only when we found that the yield that Itakura made was not enough that we—

Hughes: Can you pinpoint the date of the meeting when the light bulb went on?

Riggs: I’m not sure we can. [pause] There must be a record somewhere. [Riggs added in editing: It would be hard [to pinpoint] because so many years have gone by. But Gilbert's seminar would have been in some way on record at Caltech. Probably late '74 or early '75.]

Hughes: You began to work with the San Francisco group and with Caltech in March ’76. So obviously, you’d had the idea before that.

Riggs: Oh, yes. If that’s where you’re going, yes. This was approximately a year before that, roughly. And I think I’ll answer that partly as I continue.

So I called up Herb, and of course, he immediately saw that [the project] was interesting, so we agreed to do it. So then at some point, we got [the synthetic lac operator DNA] to Herb Boyer and Herb Heyneker. I forget whether I took it up or whether they came [here to City of Hope]. But we agreed to collaborate; we agreed to clone the lac operator that Itakura had made, this chemically synthesized gene element, in collaboration with Herb Boyer. Herb Boyer assigned the project to Herb Heyneker. Herb Boyer already had what’s now called linker DNA in his refrigerator or freezer, and so Herb Heyneker got it, devised the cloning strategy, and was successful. That work was published early in ’76, I believe.

Hughes: No, it came out a little bit later--October 28, 1976.

Riggs: Okay. So that was close enough. [laughter] Hey, if I'm within the right year—

Hughes: I know, it's pretty good. [laughs] It says somewhere in there that you began the research in March, and that's a reasonable time. You had to write up the paper, et cetera.

[End Tape 2, Side B] ##
[Begin Tape 3, Side A]

Riggs: But of course we knew the results much sooner [than October]. We knew that Itakura could make DNA that was adequate for cloning purposes. We knew that the DNA was functional in vivo. That in itself we thought was a major milestone, because this was really the first time any chemically synthesized DNA was shown to have an in vivo function.

Hughes: And you did that by binding the repressor to the operator.

Riggs: Yes. We were able to devise the in vivo assay to show that the operator was binding the lac repressor inside the bacterial cell. We knew that it was looking good. We had recognized—actually invented (nobody thought of patenting it) linkers at that time. [laughs] It's just so incredibly useful for recombinant DNA work that it immediately became standard practice.

Hughes: You mean linkers different than Scheller's?

Riggs: Yes, because Scheller, working for Itakura, made the DNA that was part of this.

Okay, so we knew that Itakura could make DNA that was functional in vivo. We were closely working with Herb Boyer and now Herb Heyneker, so we knew we had—I want to say that the recombinant procedures were—Itakura and I, starting back in the summer of '75, started at that point saying, "What's next? Now that you can make reasonably decent DNA, what's the next step?" Itakura says, and I believe him, that he was thinking insulin years prior. So he was thinking insulin. Actually, I was starting to also look into hormones, or short peptides, actually. It wasn't that we particularly wanted hormones; we wanted a short peptide that had a biological function, and hormones seemed to be a good possibility. So both Itakura and I were thinking and discussing among ourselves what hormone should we go for.

At that time, a paper came out on somatostatin, and the paper was from Roger Guillemin's lab. Then Dr. Guillemin came here and gave a seminar in roughly that time period. I got very interested in somatostatin. I said, "Oh, this is great, because it's much smaller than insulin; it's much easier to assay for; it's got a single peptide chain; it spontaneously forms an active structure, and there's a

good antibody so you can have a radioimmune assay that's extremely sensitive." So I started talking with Itakura, saying, "This is a great project, and if we only make one or two molecules per cell, we can detect it using this antibody method."

Hughes: Now, was that Wiley Vale's—?

Riggs: Wiley Vale wound up helping us, yes. I think he was on the publications.

Hughes: Had he invented the assay?

Riggs: He could have, yes. I don't know if I'd say invented; he probably did develop the radioimmune assay. He was an associate of Roger Guillemin at the time, I think already becoming semi-independent. I don't remember now that relationship too well.

Hughes: [scanning published paper] Wiley Vale is not on the publication.

Riggs: He's not on the publication. We used the published method. He was helpful, though. I did call him up, talked to him on the phone, and he was very helpful in helping us set up the radioimmune assay and just giving us information. So he freely gave advice on how to do it.

Hughes: Right. May I ask you one more thing about this earlier research, which I see—and please tell me what you think—as being critical to get you thinking about somatostatin, or at least some biologically functional and useful substance. I don't know if you were thinking commercial yet.

Riggs: None of the group here was thinking commercial yet, no. But thinking about some demonstration that bacteria could be used as factories to make proteins, yes.

Hughes: Yes, that was a big step. I notice there are a lot of authors on this lac operon paper. One of them is Narang. Now, why did he come into it?

Riggs: Itakura started working on the lac operator when he was in Narang's lab, and so we must have used some of the DNA.

Hughes: Yes. And then Rosenberg and Dickerson are the x-ray crystallographers. But did you do crystallography?

Riggs: No, never did.

Hughes: Then why were they on the paper?

Riggs: Well, we were all part of the group, and it was a direct result of our collaborative interactions. I think it was both normal and the correct thing to do.

Hughes: Then the last author is your colleague, I believe, Dr. Lin?

Riggs: Yes.

Hughes: What was he doing?

Riggs: He did the characterization work. When we checked out the DNA that Itakura made and measured the binding of the lac repressor to the lac operator using the filter assay, Dr. Lin did that work.

Hughes: Well, shall we stop for today?

Riggs: Seems a good place to stop.

[End Tape 3, Side A] ##

Interview 2, January 13, 2005

[Begin Tape 4, Side A] ##

Hughes: It's the second day of the interviews, and Mr. Goldsmith is with us again. Last time, we talked about the work that lead up to somatostatin, so today let's plunge in with somatostatin. Perhaps the place to begin is, why somatostatin? Why did you choose that particular gene?

Riggs: Well, I think I may have touched on that. Itakura and I were looking for the next project after we'd established that he could make functional DNA. So we were thinking and discussing the next step, which included hormones. We also thought about other possible next steps—small peptides that we could make. A publication appeared, I believe it was in *Science*, on somatostatin, and I saw that. I looked at it, saw that there was a very nice radioimmune assay, and that [the peptide] was only fourteen amino acids. So it was the right length. It had a very sensitive assay, and it also had—at least according to the *Science* paper, and Roger Guillemin did come up and give a seminar on somatostatin—some potential for being a therapeutically or clinically relevant peptide. Which never proved out, but there was that potential.

I may be repeating myself, but one of the next steps that occurred some time in 1975 was, after we were seriously thinking about somatostatin, I went to Rachmiel Levine, who was the medical director for the City of Hope at that time, and an expert on diabetes. He was a very well-known endocrinologist and an expert on diabetes. I asked him whether somatostatin would likely be of clinical importance or medical importance. He thought about it for a while, and then he said, no, he didn't think it was going to be clinically important. But then he said, "But you should do the project anyway." He was very supportive of the project, and he was the head administrator here at the City of Hope. Without his support and enthusiasm, I don't think the project would have ever got off the ground.

Hughes: It shows some vision, doesn't it? He was an older scientist.

Riggs: He was already probably in his seventies or thereabouts.

Hughes: So molecular biology and the molecular approach would have been new things to him that he may have felt uneasy about.

Riggs: Yes. He was still pretty active; he was pretty good. But yes, it was new to him. He was not an expert on molecular biology. He was an endocrinologist, biochemist.

Hughes: But he obviously saw the potential.

Riggs: Yes—of what we were trying to do, yes. Oh, yes, he understood. He understood how important it was.

Hughes: At this point you had found that one synthetic gene functioned in vivo, right?

Riggs: Yes.

Hughes: But how sure were you as you began these experiments that a synthetic somatostatin gene would function in bacteria?

Riggs: Okay, so we had shown that the DNA would function in vivo. Nobody had shown that you could actually make a protein product. So DNA makes RNA makes protein—none of that had been done using synthetic DNA, or even using any other approach, really. So no one had taken any, what do you say, foreign, non-endogenous DNA and transfected it and got a useful product. Nobody had done that yet.

Hughes: You mean, they hadn't even tried?

Riggs: They may have tried, but as I recall, they had just made garbage. They really had not been able to get anything correct, or that could be established as correct.

Hughes: Remind me, in the lac operator work, you showed functionality just by the binding of the repressor—

Riggs: Yes.

Hughes: So no expression.

Riggs: Right, it was not transcribed; we just showed the DNA was good. We didn't show that we had all the other steps, and then, really, it was an incredibly complex process. I don't think we fully knew at the time, but we know [now] there is a large number of proteins involved in forming the promoter complex to make RNA off a gene. And then once you get the RNA made, then there is just an incredibly complex series—hundreds of proteins involved in editing and processing that RNA. And then it gets to the ribosome. How did we know that the ribosome would accept this foreign messenger RNA? There were just a lot of unknowns that nobody had checked out.

Hughes: Were you even speculating whether it would work or not?

Riggs: Well, yes. [laughs] Okay, the other answer to your question is, yes, I did think we had a good chance of getting it to work.

Hughes: Why?

Riggs: Well, partly because we—we and others—were aware that mammals might use different codons, that the genetic code or the usage of words in the language might be different. We were aware that the language might be slightly different. But we thought we might have an advantage. We didn't know one way or another, but we thought that the—well, let's say it this way: we deliberately used codons that were frequently used in bacteria. So we tried to design a bacterial gene that would work in bacteria. We didn't copy the mammalian gene for insulin. I thought that was a real advantage. We didn't try to copy a human gene and put that into bacteria. We designed a gene that would work in bacteria. So this was a totally man-made—not only man-made but man-designed—gene, and I thought that was one of the most key aspects of what we were doing, of our approach. We didn't know that it was going to be successful, but we thought we had a good chance.

Let's see. Somewhere I've talked about irrational overconfidence. [laughs] I kind of like that term. It's important that scientists have this irrational overconfidence. You kind of know that 90 percent of other people's experiments don't work, but you have this idea—well, you know, you just have to believe in your own experiments. And I think you can call it irrational overconfidence, but I think it's a necessary component.

Hughes: Because otherwise, scientists would never take risks.

Riggs: Right. Well, it's pretty easy to talk yourself out of doing anything, so I think the successful scientists do have this sort of overconfidence. But nevertheless, whatever, I knew that there were just a multitude of unknowns, and there was no guarantee that we'd be successful. But I felt we had a fairly good chance.

Hughes: The first thing that you did, I believe, was to submit a proposal to NIH, which was the logical way to go in those days, wasn't it? Apply for grant money.

Riggs: Definitely, yes. Most scientists would apply for grant money. So yes, the logical way to get money for a project was to apply to NIH, sure.

Hughes: First of all, let me ask you if the methodology that you proposed in the grant was actually what you ended up doing.

Riggs: Well, in part it was, and in part it wasn't. So certainly some of the details changed. The overall plan remained the same: to use the lac promoter, and et cetera, and chemical synthesis—all those aspects of the project stayed the same. We had to get it precisely positioned, and that this was not an easy thing to do in

those days. So the method that we used to precisely position the chemically synthesized gene did change, but the rest of the project was pretty much the same.

Hughes: So tell me what happened to the proposal.

Riggs: [laughs] It was turned down; it was not funded. One of the reasons they gave for not funding it was it could not possibly be done in the three-year time period that we asked for. Another sentence that I remember in their critique was, "This seems like just an intellectual exercise." Maybe that's the one I liked the best. They were so wrong. [laughter] By the time we got that critique, we had already agreed to go with Genentech.

Hughes: Oh, had you?

Riggs: Yes, we had. Now, if we'd received that [negative response] before we had decided to go with Genentech, of course we would have been very upset. But by the time we got that, we had already agreed to go with Genentech.

Hughes: Why did you decide to go with Genentech before you knew whether you were going to get NIH money?

Riggs: Timing, it was timing. So [Robert] Swanson was able to obtain the money and sort of nail everything down. I forget the exact date, but April, May, something like that.

Hughes: Of '76? Yes, it would have to be 1976.

Riggs: It was in '76, yes. Also, I don't mind saying, it was a definite advantage to have a good collaboration going with Herb Boyer. So that was also a real plus.

Hughes: Do you think you would have done it without Boyer?

Riggs: Well, if the grant [application] had been turned down, and the Genentech opportunity had not materialized, we wouldn't have had the money to do it. We may have tried on a smaller scale and probably would not have been successful. So I think yes, it worked out just wonderfully. I've always been very happy that we collaborated with Boyer and got funding from Genentech.

Hughes: Did your lab at that point have the cloning technology that would have been necessary?

Riggs: Well, I could do it. I was a molecular biologist, and I could learn—I had learned. So I actually had a good, you might say, understanding and knowledge of the techniques. So yes, we could have done the cloning work, but I don't mind saying, experience allows you to do it better and faster—faster is the main

difference—and so we would have been much slower in doing it. I don't think there was any doubt about that.

Hughes: It was early enough that recombinant DNA technology was still pretty much located in only a few labs, was it not?

Riggs: Yes, sure.

Hughes: It wasn't universal the way it is now.

Riggs: That's right. So we were collaborating with those that had the most experience, and not only were they experienced, but they were also very good.

Hughes: Yes, very good, and very motivated.

Riggs: That's right.

Hughes: What I've learned from all this is that people were pretty much willing to work around the clock. There was just no stopping them.

Riggs: That's right. [laughter] Oh, yes. Everybody was motivated and working hard. I feel fortunate that we were able to form that relationship, that collaboration; it worked out extremely well.

Hughes: Was it through Boyer and then Swanson that the possibility of having the somatostatin work funded came to be?

Riggs: Well, Boyer called me and said that he had a friend—as I remember, he said, "I have this businessman friend of mine who thinks he can raise money for insulin. Would you and Itakura be interested in collaborating on that project?" I said, "Well, yes, but I'm in the middle of writing a grant with Itakura to make a gene and express the gene for somatostatin, so I think we should do somatostatin first." Then, of course, I went on to explain why I thought the somatostatin was so much better. And then I think Herb Boyer understood and agreed.

Hughes: In that one phone call?

Riggs: I think so. Because what I do know is that within a short time, it was Boyer's job to convince Robert Swanson that we should do somatostatin, and that was not easy. [laughs]

Hughes: Were you present at some of those conversations?

Riggs: Oh, at some point, yes. I doubt if I was there for the initial conversations. But sure, I wound up talking with Bob Swanson, so I do know how difficult it was to

convince him that we should make this small peptide, this hormone that did not have any proven commercial value. Swanson was very worried about that.

Hughes: It was the lack of assured commercial value that worried him, was it not?

Riggs: Sure. He understood better than any of us. As a matter of fact, I had no real understanding of how precarious the funding was, [laughs] and how short a time that Swanson believed—and I think he was probably correct—that we had in order to perform. So he understood better than we did the speed with which it had to be done. He eventually agreed that the strategy was to establish feasibility, submit patents, and try to publish the work. And, of course, the other plus would be visibility, to gain visibility and credibility—credibility is a good word. So he finally decided that since we could do somatostatin quicker and with a higher chance of quick success, that was the way to go. So he finally agreed, but we had to talk to him quite a bit. I think Herb did most of the arm-twisting.

Hughes: It probably at basis goes back to background, doesn't it? I mean, he was the business part of Genentech.

Riggs: Yes, absolutely.

Hughes: And the rest of you were the science. So the goals were slightly different.

Riggs: Slightly. And he understood better than we did. No question about that.

Hughes: Well, doesn't it go back to the naivete of biologists in general about the commercial world?

Riggs: At that time, very few biologists were, you might say, interacting with biotechnology. And then, of course, the word biotechnology hadn't even been invented yet. I think it was invented at about the same time that Genentech was established. I'm actually curious about the word. I know it started appearing at about that same time.

Hughes: I can give you a reference to a book about that, because it actually is an older term, but it was revived for the new biotechnology.[3] It was a German term that was used, I think as early as the thirties, but of course not referring to recombinant DNA and DNA synthesis, but rather to using microorganisms to produce useful products.

Riggs: Oh, I see, that was called biotechnology.

Hughes: Did you have any hesitations about accepting funding from a company?

3. Robert Bud, *The Uses of Life: A History of Biotechnology*, Cambridge University Press, 1993.

Riggs: I don't remember having any serious misgivings. I remember telling the City of Hope and our—I guess now it would be called our intellectual property office and so forth, but then I forget what it was called—just letting them know I was very enthusiastic about the project. But no, I had no misgivings.

Hughes: Had City of Hope done similar deals before?

Riggs: I don't think so. It may have been the first. I don't know that, though, one way or another.

Goldsmith: I was just going to say: we didn't even have an intellectual property office at the time.

Hughes: Did anybody patent before?

Goldsmith: It was our first. I think we were unaware of any other commercial relationship that we had had. We were neophytes.

Riggs: [laughs]

Hughes: You and a lot of people in academia.

Goldsmith: At that time, these kinds of collaborations were very new.

Riggs: Now, I started out as an organic chemist. I kind of thought of myself as a chemist, at least for a while. So universities that had chemistry departments and had engineering departments and so forth, of course, they did [patent]. So the ideas were not foreign to us. It's just that the biologists and the molecular biologists didn't have the opportunity, so therefore didn't think about [patenting] very much.

Hughes: Were you or City of Hope as the institution accepting this money from a company criticized presumably by other academic biologists for "colluding with industry"? [laughter] To put it dramatically.

Riggs: I don't think that ever became an issue here at the City of Hope. I do recall that it became an issue at UC San Francisco.

Hughes: Very much so, yes.

Riggs: No, I don't remember [any criticism], and I think I remember correctly; I don't think it was ever an issue here at the City of Hope.

Hughes: I wonder why.

Goldsmith: If I could jump in: at the time, Genentech was Swanson and Boyer, period. And that's what it was.

Hughes: One of the criticisms was that there was no separation between Genentech and Boyer's lab at UCSF.

Goldsmith: Right. So the perception could have been, we were in collaboration with Dr. Boyer and his lab.

Hughes: Tell me the original plan for how you were to go about this work.

Riggs: [laughs] Well, the foundation of the plan was for Dr. Itakura to synthesize this piece of DNA that would code for somatostatin. Then we were to use the lac promoter—I like to use the analogy with a tape player—so the lac promoter was going to be the tape player. And then [with] my experience with the lac operon, the lac promoter, I thought we knew enough to correctly position the DNA that Itakura made just appropriately downstream of the promoter. But that was not easy. So in the grant application, there was an editing method that did use synthetic DNA that Dr. Itakura and I proposed—well, I think I don't need to go into the details. But there was an editing method that we think would have worked, so if a better method hadn't come along, we think that method would have worked.

Hughes: But you never tried it?

Riggs: We never got around to trying it, because of the change in plans, for example. There was one other change we'll get to. So we changed the way we inserted the gene and the way we edited it. The other parts of the process, that is, using the radioimmune assays, how we were going to assay for the somatostatin, how we were going to determine that we were getting the desired product, all of that stayed pretty much the same.

Hughes: Define edit, when you're referring to genetics.

Riggs: Well, let's see. I'll try to use an analogy maybe with a paragraph or something. A period is a stop sign, so you have to have a way to stop the transcription. You have to have a way to start the transcription, and sometimes you have to lengthen the DNA, but we made it chemically, so that wasn't a problem. But you have to make sure that the start site is exactly right, and then you have to put that into the cassette player so that it fits in exactly right. Maybe that's a good analogy: you have to have it fit in exactly right. The editing was where the recombinant DNA technology really came into play and allowed us to do that. And there was a synergism between the chemically synthesized DNA and the recombinant DNA. Come to think of it, that's one of the most important aspects of what we did. And then later it became the key advantage that Genentech had—the synergism between the recombinant techniques and the chemical DNA

synthesis. Put them together and you can do just about any editing job that needs to be done.

Hughes: In the early days of Genentech, that's what moved it ahead of any other competitor, wasn't it?

Riggs: It was extremely important; it was extremely important.

Hughes: You couldn't have done this early work without the conjunction of these two techniques?

[End Tape 4, Side A] ##
[Begin Tape 4, Side B]

Riggs: We definitely had to use the recombinant DNA technology, sure.

Hughes: What was Boyer's role in this? He does appear on the paper as an author. How did he contribute?

Riggs: Well, he was the head of the laboratory, and he had the best lab in the country, perhaps, in doing the recombinant work. He contributed Herb Heyneker and also Paco Bolivar. Maybe I should say Francisco Bolivar; everybody called him Paco. Boyer and I and Itakura probably ultimately made all the managerial decisions on the project. Itakura made the managerial decisions in his group, which was of course mostly focused on the DNA synthesis. But we also consulted Boyer in all aspects of the project. So Herb and I and Itakura made all of the managerial decisions on the project. I think Herb's contribution was extremely important and [his name] absolutely should have been on the paper.

Hughes: So what went wrong with the first approach? [laughter]

Riggs: With hindsight, I can say that we underestimated the vigor and efficiency with which *E. coli* can degrade small peptides. I argued in favor of making it with the short fusion peptide, but later on we moved it back towards the end of beta-galactosidase. We did know that both options were available. But I argued that the more forward position, making the short protein, was better because I thought it would work. Even though we were aware that degradation might be a problem, I didn't think it would destroy all of the molecules. I thought we'd still be able to detect it. And I thought our chances of getting a high yield was actually reduced by having all of this beta-galactosidase protein, which is a huge protein. My thinking was, you don't really want the bacterial cell using up all its energy making this huge protein which you're later going to throw away. So I did argue for the short version first.

Hughes: I see.

Riggs: I was wrong. [laughs] I was wrong. I thought we could detect one or two molecules of somatostatin per cell and declare success and then worry about the yield later on. We didn't even get that.

Hughes: Did your heart sink? How did you react?

Riggs: I was not happy. I was not happy at all. I still had other projects that were funded, so I had not devoted all of my future to the somatostatin. So I was very crestfallen and unhappy, but I don't think I was as emotionally affected as much as, say, Robert Swanson or others, or maybe even Herb Heyneker. They had more of their—people tend to—science is their life, so to speak. So I never thought my life was ending. [laughs]

Hughes: Well, Swanson in a sense was right. His company he saw going down the drain in a very real sense.

Riggs: Right. Oh, yes. He was quite upset.

Hughes: How did he happen to be there?

Riggs: We had everything ready to do the assay, and we let the group in San Francisco know and let Bob Swanson know. So they all came down to be here to celebrate.

Hughes: Paco and Herb Heyneker and Swanson?

Riggs: Gosh, let's see. You know, I don't remember one way or another whether Paco was here. He may have already gone back to Mexico. But certainly Bob Swanson came down, and Herb Boyer came down, and Heyneker must have been here, too. Swanson stayed in my house.

Hughes: So you got a heavy dose of how he was feeling.

Riggs: Yes, I know how white he was, deathly white, in the morning after the failure.

Hughes: Is it true that he checked himself into a hospital?

Riggs: Yes. Turned out to be just acute indigestion, but we were worried for him.

Hughes: Yes, I'll bet you were. Well, you talk now about efficient degradation, but did it ever occur to you that it wasn't going to work at all? That this scheme that you had of producing mammalian proteins in bacteria just wasn't going to work?

Riggs: We still had things to try, so we had plan B, and we also had plan C. [laughs]

Hughes: What were they?

Riggs: Well, plan B was to move the somatostatin gene back to the tail end of beta-galactosidase. So we had that to try, and that was the one that we shifted to. Plan C was one that I actually thought was a really good idea, but we never got to try it out, so I don't know whether it was or not. But I thought we could use a puromycin. Because somatostatin was so short, I thought we could inhibit protein synthesis by using puromycin and sort of overwhelm the degradation system. So I had this plan of adding this protein-terminating drug at the same time we induced, and it might have worked.

Hughes: You had this all thought out before you began the experiments?

Riggs: Yes. That's what I remember, yes, definitely.

Hughes: What was the interval between that heart-stopping moment when it didn't work and the moment of success?

Riggs: Things went pretty quickly, and we were lucky in that the second site didn't require any further editing, so we could just cut and paste in the somatostatin gene. Also the amino-acid sequence of beta-galactosidase became known, so we could use that. So that all fell together nicely. I think it only took about three months, two months, something like that. Heyneker actually did the cutting and pasting, and it didn't take him very long. And we had the radioimmune assay going. Louise Shively and I worked on the radioimmune assay, so we had everything ready to do the assays. So I think it was just a few months later that we were ready to try again. [laughs] And this time, and I fully understand it, Bob Swanson didn't come down. My speculation is that he was just too nervous to come.

Hughes: Did any of the Genentech people come?

Riggs: I think it was just Itakura and I. Yes, I think that's correct. Maybe Louise Shively was there too, but we did get some pretty encouraging results. They actually looked real good. So we were very happy, shook hands, and as I remember, it was late. I was supposed to take my son to a Dodger game that evening, and so I think I had to leave quickly or else I would stand up my son. So I think the thinking was, well, it looks really good; we've probably got it, but let's not overreact until we can make sure we can reproduce it. So that was kind of our thinking.

Hughes: Did you call Genentech?

Riggs: Probably yes; I don't remember. What I remember is going to the Dodger game. It was at Dodger Stadium, and I remember going there, but I don't remember anything about the game. [laughter]

Hughes: You were thinking about the research.

Riggs: I was thinking about all the incredible possibilities now that were likely to come to be. It's a spectacular setting. It's a beautiful stadium, and so it was not a bad place to be, to have your mind elsewhere, sort of contemplating miracles.

Hughes: Were your miracles mainly commercial? I mean, producing therapeutic proteins?

Riggs: Well, I'm not sure I would use the word commercial. Medical—you know, curing diseases and practical applications, kind of early on I was thinking about. I don't know whether it was that night or not. But early on, I was thinking about improving the production of enzymes. A whole world of opportunities suddenly became, let's say, much more likely. You still didn't have certainty for a lot of things—maybe it was no longer necessary to be irrationally overconfident. Now you could just be confident that you could solve any remaining problems.

Hughes: Swanson was not around, so you couldn't have had that conversation. But did you give any thought to the future of this small company? Did you think, well, maybe now Genentech has a chance? Did your mind go in that direction at all?

Riggs: I'll answer that by saying that I had the mindset that if the company was successful, this would provide a funding mechanism for basic research. So I was feeling optimistic that Genentech would be able to fund further work here at the City of Hope. So I did think in terms of success of Genentech, but I was thinking in terms of then being able to get contracts and whatever to continue working with them. I was thinking along those lines.

Hughes: I remember Herb Boyer saying something similar. The first money that he accepted from Genentech which would have had to have been for the somatostatin, wouldn't it?

Riggs: Yes.

Hughes: He had a similar idea, that this was a new source of support for his laboratory.

Riggs: Yes, that's right.

Hughes: Which makes sense; that's a big concern for scientists.

Riggs: Well, that's one of the major jobs. A big part of your job as a principal investigator or the head of a laboratory is to somehow raise the money to fund your research. So I was definitely into that, I was thinking that now Genentech would be a nice source of funding for the research that I wanted to do.

Hughes: There are two people that we haven't mentioned in connection with somatostatin, and one of them is Tadaaki Hirose. He was in your lab?

Riggs: No, he was in Itakura's lab.

Hughes: So he was helping with the DNA synthesis?

Riggs: Yes. He was an important postdoc; I think he was a postdoc in Itakura's laboratory.

Hughes: I see. And then at some point in 1977, Roberto Crea arrived. He too went to Itakura's lab.

Riggs: Yes.

Hughes: What was his particular contribution?

Riggs: Well, let's see. He was already experienced in DNA synthesis. I don't think I remember exactly what his contributions were, but he was a very important member of the team.

Hughes: He had expertise in a new machine called the HPLC.

Riggs: HPLC, that makes sense.

Hughes: What does that stand for?

Riggs: High pressure liquid chromatography.

Hughes: I get the insulin and the somatostatin research a bit mixed up. But I know on the insulin project that purification was one of the roadblocks, that that caused some of the problems. Was that also a problem in the somatostatin project, and is that maybe what Crea contributed?

Riggs: Let's see. I think he came towards the end of the somatostatin project. He may have been part of it, but I think he came pretty much toward the end of it.

Hughes: Yes, I know he wasn't there in the beginning.

Riggs: I think he did help the insulin project go forward faster, and it would make sense—so I think it's probably right that he brought the HPLC expertise with him. Yes. Let's see—David Goeddel and I went with him at least one time. I think we used the HPLC over in Itakura's lab, and probably we got Roberto's help on using the HPLC to purify one of the chains of insulin, either the A or B, I forget which.

Hughes: I know B was the one that was giving trouble. Now, word got to a subcommittee in the U.S. Senate—maybe I should back up a bit. One of the things that was going on in the larger scene was the recombinant DNA controversy.

Riggs: Yes, that's right.

Hughes: Which by 1977 was pretty hot because bills have been introduced in Congress, and there was state legislation pending, and also local ordinances—all kinds of thingslegislation at all government levels. The opponents of recombinant DNA were pretty vocal. Tell me about the incident where it was announced in a Senate subcommittee that a small company and City of Hope and UCSF have succeeded in expressing a gene.

Riggs: Well, let's see. You know, I wasn't there.

Hughes: In the Senate, you mean.

Riggs: That's right. Let's see, the person's name—?

Hughes: Handler, Philip Handler, who was president of the National Academy of Sciences.

Riggs: Yes, I do remember his glowing statements, which I agreed with. [laughs] I don't think anybody really expected this to be successful so soon. So it was—yes. And these hearings were going on, or had been going on, and so it was quite newsworthy, definitely.

Hughes: Do you know how Handler knew of the work?

Riggs: Not first hand.

Hughes: I just wondered if somebody called him.

Riggs: I don't know one way or another.

Hughes: When was the paper published?[4]

Goldsmith: I think it was a result of the paper.

Hughes: The publication had come out? [looking through papers][tape interruption]

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4. The paper--Itakura et al., "Expression in *Escherichia coli* of a chemically synthesized gene for the hormone somatostatin," *Science* 1977, 198:1056-63—was published on December 7, 1977. The announcements—by Paul Berg as well as Handler—in the U.S. Senate subcommittee occurred on November 2, 1977.

Well, maybe you could talk about the publicity around the somatostatin success.

Riggs: Yes, there was a lot of publicity. It was very well received as newsworthy and even comments from testimony before the Senate. So it was a big thing, no question about that.

Hughes: Did that surprise you, how fast the word spread and how big the reaction was?

Riggs: Not really. I thought it was important, yes. I thought it was important.

Hughes: Were you thinking about the recombinant DNA controversy at all, and how the work on somatostatin might relate to the political problem?

Riggs: Well, we were—yes, I would like to say. We were very much aware of the controversy, and the controversy actually influenced a change in the plan. The original plan was to produce somatostatin without any precursor protein at all. I like to think that one of the key inventions—and of course, I'm biased, because it was my idea—was deliberately putting a precursor protein in front of the desired product. I think that was a novel idea at the time. And that came about partly because we ran into another problem that I won't go into the details. But the promoter construct that we thought we had wasn't quite right, and so we did have to think about it. One site that we could put it into resulted in, I think it was, eight or ten amino acids of a precursor protein. In thinking about that, I suddenly realized that this is a tremendous advantage because we can make [somatostatin] as an inactive hormone. I had of course done a lot of reading on somatostatin, so I knew that the N-terminus—as it synthesized the left end—was very important for activity. So if there was a precursor stuck on there, the hormone would be inactive. So that was a sort of light bulb: this is a tremendous advantage. But of course, now you have a product that has a precursor; you have this worthless product. But the second part of that idea was that because the start codon is a methionine, an ATG—I won't try to explain it, but anyway it results in methionine in the product. I knew from previous work I'd done on beta-galactosidase that you could cleave very specifically at that methionine. So you could cleave off the precursor—

Hughes: All right, so that wasn't going to be a problem.

Riggs: So you can make an inactive product. And then in the test tube, there's this really nice, efficient cleavage [method] where you could cut it and generate the desired active product. And that was immediately—everybody thought, yeah, that's the way to go. So the team just jumped on that, and that became our plan from then on.

Hughes: Tell me about the press conference, and how that related to the paper that was coming out.

Riggs: Well, let's see. [laughs] Of course, that was my first experience with any newspaper type of publicity, and television also.

Hughes: Was the press conference arranged by Swanson? Was he the one pushing it?

Riggs: Well, he certainly was one of the people. But our own City of Hope public relations department was also excited about it. I don't have any knowledge about UC San Francisco, but I'm sure they also were not unhappy about it.

Hughes: Oh, I'm sure. There is a UCSF press release; I don't think I have it with me.

Riggs: So it was recognized by everybody as being a really major breakthrough, a real milestone.

Hughes: Was there any debate about where the press conference was going to be? Genentech did not yet have facilities.

Riggs: That's right.

Hughes: So I guess the only other place it possibly could have been would have been at UCSF.

Riggs: That's right. You know, I don't remember. There must have been a decision one way or another, whether here or there. But I don't remember now how that was made. It did wind up being here at a hotel in downtown L.A. I don't remember how that was decided.

Hughes: The paper, as we just discovered, was published in early December, 1977. So presumably, the press conference happened a little bit before that.

Riggs: I wouldn't dispute that. What I do remember is that we—both Herb Boyer and I—insisted that we wait until the paper was accepted [before we made an announcement]. So what I'm remembering is that we wanted to have the work go through the peer review process. So we were advocating waiting for the standard procedure. Swanson was, of course, very eager—it had to be [announced] immediately. I think the compromise that we reached was when the paper was accepted, then we could have the press release. We also talked about it publicly. Was it Itakura that gave a seminar at UCLA? I also can't keep my memory straight between somatostatin and insulin. But for one of those, Itakura gave a seminar over at UCLA.

Hughes: So that implies that a patent had been applied for.

Riggs: Oh, absolutely.

Hughes: When did that all occur? Was it even before you'd begun the experiments?

Riggs: No, the way it usually works and the way it did work was that as soon as you have the results, as soon as you have the experimental data that seems to be the basis of a patent, then you take that information to a patent lawyer. Then the patent lawyer, who understands the law—what scientists think doesn't really matter; it's the patent law, and it's the patent lawyer that understands that. So we did that. So Swanson hired one of the best patent lawyers that he could find, and that happened to be Tom Kiley.

[End Tape 4, Side B] ##
[Begin Tape 5, Side A]

Hughes: Who was working with Kiley? You and Itakura mainly?

Riggs: Eventually, Kiley talked to all the scientists. And we also had a group meeting where we all got together and discussed it. We prepared a draft of the paper—and this is sort of standard practice, then as now—and we gave that draft to Tom Kiley.

Hughes: A draft of the patent application?

Riggs: No, of the paper that was going to be published. So he would look at that, and then he also got additional information from talking to the scientists. Rather quickly, he wrote the patent [application]. That's the way it's always done; the patent lawyers are the ones that write the patents.

Hughes: Why, with all these people involved, were you and Itakura singled out as inventors?

Riggs: Well, first of all, it was Tom Kiley's decision. He investigated and then decided on inventorship based on the patent law. Might as well get to the real question: why were Herb Boyer and Herbert Heyneker—why were they not inventors?

Hughes: Right.

Riggs: Well, the reason is that, even though their work was extremely important, the recombinant DNA—and most, essentially all of the recombinant DNA work was done by Herb Heyneker in Herb Boyer's laboratory, and Herb Boyer was part of the design. So I was actually an advocate saying that they should be inventors. I really did; I thought they should be; we were collaborators. I argued with Tom Kiley. But he pointed out that what they did was using the same techniques that they had published a year before, so it was already published. So what they did was important, but the technology had already been published, and so it was already public knowledge. The patent laws are such that they then—what they did on somatostatin was not, could not be considered an invention. So Tom Kiley carefully investigated about what was novel and inventive about the

project that hadn't already been published previously, and it turned out that it was Itakura and I that had made the new invention.

Hughes: Now, I can understand you being an inventor because you just explained in some detail the three plans which you had thought out. But what was new about what Itakura was doing?

Riggs: Well, the key on that was that he and I developed the somatostatin plan. That was important. It turns out we were the first. We were the first to write down a concrete plan with sufficient detail to support a patent, and to support the idea that an invention was made. Itakura and I were the ones that wrote and planned the NIH grant application.

Hughes: I see. That made a big difference, then, didn't it? Because that was concrete evidence that you'd thought this out, and there were dates associated and all that.

Goldsmith: And if I could just jump in to say, you may have gotten this from Kiley, but Kiley made the determination that the invention was conceived in the NIH grant application, and that was the date of the invention, and that was prepared jointly with Art and with Keiichi Itakura. This was Kiley's determination. And there also were separate patents issued solely in Dr. Itakura's name. So he came up with other ideas as part of this collaboration that were solely his. But the principle invention was that described and conceived as reflected in the NIH application.

Hughes: I see.

Riggs: Yes, and Swanson hired a very good patent lawyer. The scientists were naïve—we didn't know what was an invention and what wasn't. And like I say, we did—not vehemently—but we did argue with Tom. I remember distinctly arguing, and he had to explain to us that this could not be considered an invention; this could be, and so forth.

Hughes: Now, this was Kiley's first patent in biotechnology. It would have to be.

Riggs: In biotechnology, yes.

Hughes: Yes, because this was the beginning of modern biotechnology. He did not have a science background.

Riggs: I think he did.

Hughes: I take that back—he was a chemical engineer. But he didn't have biological training, and, specifically, he didn't have prior knowledge of the techniques that you were using. How did you work with that? How did you have him understand the science?

Riggs: Yes, well, let's see. One of the things we did was give him a book written by Jim Watson, I think, *Molecular Biology—*

Hughes: *—of the Gene*.

Riggs: Yes, I think it was *Molecular Biology of the Gene*. I was actually very impressed: Tom Kiley was a very quick study. He quickly grasped the essentials of what it was all about. The answer is that he learned quickly.

Hughes: So with you two as inventors, both City of Hope, the application then went through City of Hope without any—no?

Goldsmith: [interrupts] No. City of Hope assigned its interest—

Hughes: Oh, to Genentech, of course.

Goldsmith: And Genentech prosecuted the patent.

Hughes: And that was because of the way the contract was drawn up?

Goldsmith: Correct.

Hughes: Well, maybe we should talk about the contract, then, which must have preceded everything, did it not? You had to have the contract in place before you could begin—

Riggs: Before we accepted money, yes.

Hughes: And were you involved with the contract?

Riggs: No. I mean, yes and no. I was involved in communicating to the City of Hope—it was Eli King; Eli King was our research administrator at the time. So I communicated to him that I wanted to collaborate and have this contract with Genentech, so I did communicate that to him. But no, I was not involved in negotiating the contract.

Hughes: So who was?

Riggs: Well, Eli King, and he sought legal advice. I forget now the name of the person—

Hughes: Oh, I saw the name—John Hall.

Riggs: Right.

Hughes: Who was a City of Hope attorney?

Goldsmith: He was an outside patent lawyer that City of Hope hired.

Hughes: Which again tells you something about the state of intellectual property, doesn't it? The fact that there wasn't a patent attorney on staff..

Goldsmith: There's still not today.

Hughes: There's still not? Oh, is that so? [laughter]

Goldsmith: When you have a broad base of technology, it's difficult to have one lawyer who has expertise in all the different disciplines, so one goes to a patent law firm where you have a variety of patent lawyers with different scientific backgrounds.

Hughes: Was there anything unusual about this whole procedure as far as you could pick up from City of Hope?

Riggs: Unusual, you mean—?

Hughes: Well, we talked a little bit before about an academic or a research institution accepting corporate money.

Riggs: Oh, okay.

Hughes: I mean, had City of Hope experience in dealing with research contracts?

Riggs: I don't think so. I think that Eli King and those that he was seeking advice from, I think they were kind of inventing it as they went along, you might say.

Hughes: Well, what should we say about the contract? The contract, as you've said, gave rise to Genentech, and then there was a royalty clause, which probably we don't need to go into. But because it was the earliest contract of this kind, to my knowledge, how would you like history to remember it?

Goldsmith: You know, it was. It was the first agreement into which City of Hope had entered, and it was probably one of the earlier agreements of its type, at least in biotechnology. And it was an interesting agreement. It was a combination of a collaboration agreement, because it wasn't just, we license you certain rights; it was, we are going to work together. It originally provided for three years, and then was extended for five years, in which the parties anticipated that they were going to work on different proteins.

Hughes: So that was anticipated?

Goldsmith: Yes.

Hughes: It wasn't geared just to somatostatin.

Goldsmith: No. Well, Art can talk to this better than I, but again, somatostatin was the proof of principle. The parties knew that the first really productive target was most likely going to be insulin. And so there were different aspects. City of Hope, just so you understand the contract, was going to synthesize the DNA for somatostatin, insulin, and eventually growth hormone.

Hughes: Did it say so? It named those proteins?

Goldsmith: I believe the original agreement provided for insulin and somatostatin. I believe it was the amendment that included growth hormone—I can't recall whether it was in in the beginning. And so there was that part of the contract, that Genentech was going to pay to fund that research, and then from any inventions, patents that resulted from the research, Genentech would pay a royalty on a going-forward basis. So they funded the research making the genes in the research, and then to the extent it was successful and there were patents, Genentech would pay a royalty over the life of whatever the patents were. In general, that's a broad overview.

Hughes: Is it unusual to have in one contract the collaborative aspect and the intellectual property part?

Goldsmith: Today, it's much, much more common to have a collaboration agreement that provides, if something comes out of it, then the parties will negotiate a license agreement with royalty terms. Since you don't know what you're getting. And depending upon the nature of the institution, if it's a nonprofit institution and there's certain aspects of it, you in fact can't really agree in advance on what the royalty is going to be until you know what you have. So today you would usually see it in two agreements, where in this [contract] it was somewhat of a hybrid.

Hughes: Were you being told about all this, or was this not particularly of interest to you?

Riggs: At the time?

Hughes: At the time. I know it became of interest— well, forced interest—at a later date.

Riggs: No. I was—

Hughes: You were letting them handle it.

Riggs: I was not involved. I would just say that I was thinking in terms of a continuing source of funds for my laboratory. I did follow through on that, and I did successfully talk Genentech into supporting work initially on some vaccines,

using the precursor-peptide approach, and then later on with antibodies. So we did get funding from them.

Hughes: Was growth hormone at all in your mind at the time of somatostatin? Were you thinking that far ahead?

Riggs: I don't remember one way or another. It could have been. I do remember that I didn't really want to work on growth hormone, and I didn't. I wanted to work on the other applications of the technology. The first one I started on was vaccines. I wanted to work on using the precursor peptide to stimulate the immune response. I thought that's what I wanted to do, and I figured that somebody else could do the growth hormone.

Hughes: Let me go back to the contract question, because Genentech also had a contract—I think we mentioned it yesterday—with Caltech, which presumably didn't go anywhere, because Caltech and that Scheller episode that we talked about ended up not contributing to the somatostatin. So did Caltech drop out of the picture at that point?

Goldsmith: I can't remember. I remember there was some agreement with Caltech, but it was—

Hughes: There was a contract.

Riggs: I don't remember seeing it. I'm sure Bob Swanson handled that.

Hughes: Yes. Well, there was also a contract between Genentech and the UCSF group. That's where Boyer's [research] money came from.

Riggs: Yes, I'm aware of that.

Hughes: I've never seen the City of Hope contract. Have both of you seen both of them? I'm just wondering how different those contracts are.

Riggs: I have not seen the UC San Francisco contract. So I don't know.

Goldsmith: And I don't recall having seen it. I may have during the course of the past many years, but—

Hughes: Do you remember the date when the City of Hope contract was signed?

Goldsmith: It was, I think, August 1, 1976.

Hughes: It was August ’76 for the UCSF contract, which I have seen. I don’t think I have it with me. So the two contracts happened at the same time, essentially. Swanson was out there getting this thing going, wasn’t he?

Goldsmith: Right, yes. Because Genentech was incorporated in early 1976.

Hughes: Yes, April.

Goldsmith: So whatever [Genentech] did, with both of us, had to occur after April and presumably by August.

Hughes: Yes. Was it always thought of in terms of multiple patents rather than—

Goldsmith: Yes. The claims changed over time. New claims were added over time that related back to the original work. And so as people thought of things, the patent lawyers, Kiley particularly, thought of better and broader claims, and those resulted in additional patents. I think there were eleven United States patents, and I think as we said—if my number is right—126 patents outside of the United States. Something north of one hundred. [laughter]

Riggs: I don’t think I ever kept track of those that were actually non-U.S.

Goldsmith: I’m sorry, at least at the trial, it was 127 patents worldwide, and eleven U.S. patents, in either Dr. Riggs’ name, Dr. Itakura’s name, or both of their names.

Hughes: And 127 includes the U.S. as well?

Goldsmith: Worldwide, yes.

Hughes: Well, that’s quite impressive. In 1982, the first patent that comes out, and they’re all being issued at different times?

Goldsmith: I think the first one was around ’82, and the last one of which I’m aware issued I think in 1999.

Hughes: And were they generally conceived to cover a generalizable technology? Unlike the contract which as we’ve just said was aimed at specific proteins, this was a method patent?

Goldsmith: Actually, the contract was aimed at a broad method. Plus, that it was going to be established through the development of a couple of specific [proteins or strands of DNA][5].

Hughes: I see. So that’s a little different, isn’t it? You’re putting the methodology first.

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5. Goldsmith added the bracketed phrase in reviewing the transcripts.

Goldsmith: Right. The methodology was the most important. The specific DNA strands were to show that it would work, and that's what the certain dollar funding was to make these genes. But the idea was to develop a general method.

Riggs: A word that was and still is used is feasibility. So somatostatin was a demonstration of feasibility of the whole—I remember very clearly thinking that this was just to demonstrate that we really knew enough about the way a bacterium works, and that we knew enough about making genes that we could get the whole thing to work. So it was a demonstration of feasibility which had very, very broad implications.

Hughes: Yes. And, of course, you had the information from Dr. Levine—a man you respected—who had told you that he doubted that somatostatin would have much therapeutic use. So on several counts, that wasn't why you were doing [the experiments].

Riggs: That's correct. I would then confirm that. Yes, nobody, maybe even including Swanson but especially Swanson, nobody thought that somatostatin would have any commercial value in and of itself.

Hughes: Well, can you think of anything more to say about somatostatin?

Riggs: I think we've covered most aspects of it. So no, I think we're good.

Hughes: Let me see. Here I have a note: August 1, 1976, Genentech and the Regents of the University of California signed a sponsored research agreement regarding "in vivo synthesis of various polypeptides including synthesis of somatostatin and insulin."

Goldsmith: So the agreements were dated the same day.

Hughes: Is that so? That's interesting. "UC to earn royalties on patentable developments."

Goldsmith: Yes. So they were both—the royalty base was whatever patents issued, and the patents were broad method patents. But again, the specific genes were described because that's what people were focused on. Insulin being—I think at the time, Swanson knew that's where he wanted to go. But Art persuaded them that it was smarter to build a smaller gene first.

Hughes: You very wisely brought in, when we were talking about the somatostatin research, that this wasn't the only thing you were doing. When you get into this history, it's hard to realize that there is a world out there that is beyond somatostatin and insulin. [laughter] So was this still true all the way through, that yes, you had this contract and you had this interest that was leading you towards human insulin, but you were doing other things? Or not?

Riggs: Well, I had this problem that I was funded by NIH to do work on X-chromosome inactivation and lac repressor. So I was obligated to do other work. Most of my time was probably spent—I should say the majority—the majority of my time was probably spent on the somatostatin project. I kind of let the others go a little bit, but I did have other people in my laboratory who were not working on the somatostatin project. You might say I was obligated to keep the other projects going.

Hughes: You are implying that the somatostatin took pride of place, for a while anyway.

Riggs: Oh, yes.

Hughes: But this is probably true of any research when you have multiple research projects going.

Riggs: You have to strike a balance. It's good to have several irons in the fire, as long as you don't get spread too thin. So yes, it's standard practice to have more than one project going in the laboratory.

Hughes: How soon after somatostatin did the work on human insulin begin?

Riggs: Almost immediately. I don't remember exactly, but in effect, it was, well, we celebrated one night and started working the next day.

Hughes: Oh, really! [laughs] That's remarkable.

Riggs: Yes. I think that would be not an exaggeration.

Hughes: Were you aware that human insulin in many ways was a different story than somatostatin? It was a terribly competitive situation. Or at least, that's how it's portrayed in the book that has been written about the subject.

Riggs: I was not fully aware of it, but yes, I did know that there were other groups working on it, both at UC San Francisco and then at Harvard, the Wally Gilbert lab. Yes, we knew about that. Bob Swanson actually used it as a motivation factor.

Hughes: Oh, did he?

Riggs: Yes. Oh, yes. [laughs] Well, he was both genuinely concerned about the competition but also trying to motivate us. So we were aware of the competition. And I did feel that there was competition, although my position that I did actually voice was, "Well, wait a second. We're doing something different. We are doing this chemical DNA synthesis, and our approach is different. So we'll be able to publish anyway." [laughs] Well, that was not the correct way to talk to Bob Swanson, because [laughs]—

Hughes: He wasn't so interested in publication; he was interested in a product.

Riggs: His company would fail if we didn't get there first. So I was probably not politically correct in talking about that. But I did feel that there was a fall-back position. Nevertheless, I am competitive, and I did want to be first. So yes, we were in a race; we knew that.

Hughes: As a result, did you put more pressure on the insulin work than you might have if there had not been this race?

Riggs: Well, actually, it's not only true of Itakura but also most scientists, especially if they're principal investigators: if they've got their own laboratory, they are motivated, and they're already working as hard as they can. So for me to ask Itakura to work harder, put in longer hours, would have been counterproductive. It was counterproductive for Bob Swanson to put pressure on Itakura and me, and I think it's probably also true for others.

Hughes: You found it irritating?

Riggs: Yes. But I don't think he knew that. And I don't know that he ever really understood that we were already working as hard as we could. Everybody has their limit. So we were all working at the limit of our capabilities.

Goldsmith: May I just interject a minute, just to ask a question that might direct where you go?

Hughes: Yes, sure.

Goldsmith: I always viewed the somatostatin work as part of the insulin race. At the time that Art and Boyer were working on somatostatin, they knew that was part of getting to insulin first, so I'm not sure that once they got somatostatin, they accelerated their pace. The pace was already intense, because, again, I don't think you can divorce somatostatin from the insulin race.

Riggs: Yes, I would agree, and I would just say yes, we were all working as hard as we could. Often coming in at night—when necessary, coming in at night.

Hughes: I would say there's one difference, perhaps, in the acceleration, and that's when Dave Goeddel comes on board at Genentech. He sounds like a fanatic.

Riggs: Yes.

Hughes: He talks in the oral history of coming down to City of Hope with Heyneker, who's supposed to be looking for a house, but he gets roped into working on human insulin. [laughter]

[End Tape 5, Side A] ##

[Begin Tape 5, Side B]

Hughes: Goeddel described how one would sleep and the other one would get up to work, and how they worked on the technical aspects of it, trying at every juncture to make the research go faster. I'm not quibbling with the fact that you and Itakura were going fast, but I think these two crazies were probably working even more ridiculous hours and at a faster pace.

Riggs: [laughs] Yes, I would agree with that. Well, I might just add some information. We were short-handed for the molecular biology-recombinant DNA portion of the project. Genentech didn't have laboratories at the time, and the DNA synthesis was progressing well. It was just Louise Shively and myself, and I guess we had another technician. But quite frankly, I was not very good or very efficient in the laboratory, so I didn't really count as a person to actually physically hands-on do the experiments. I actually tried, and found that I had lost sufficient dexterity that I actually couldn't. There was a time that I remember I offered to help, and it was a shock to me to find that I really couldn't.

But we were short-handed, and so Swanson, Boyer, everybody were really looking for people. So I remember that being able to get Heyneker was really a stroke of luck, to get him back from Holland. Then I later learned how good Dave Goeddel was. Now, I think I actually said earlier that everyone was working as hard as they could, and everybody had different limits. Also, even in efficiency, just in terms of getting experiments done, some people are better than others. David Goeddel is one of those people that is just incredibly good. He thinks well, but more than that, he has the energy to work all through the night. So he'll go for twenty-four hours at a time and get by on very little sleep and not make mistakes. Most people that try that, their physiologies really wouldn't let them. They'd make too many mistakes. But Dave was able to do that. I think he was able to push Herb Heyneker into an incredible effort also. So I confirm without any hesitation that Dave Goeddel was a very good, very key asset that came in and helped us finish the insulin project, no question.

Hughes: Well, speaking again simplistically, you could divide scientists into two rough categories. You've made it clear that you fall on the theoretical, conceptual side.

Riggs: Or managing or designing.

Hughes: But not the golden hands in the laboratory, where Dave has been called the scientist with the golden hands.

Riggs: Yes.

Hughes: Heyneker has also said too that he really liked doing experiments. That's what he got his thrills from, the actual manipulation of experimental material. So you've got two different orientations to science, I think.

Riggs: Yes, sure, and all combinations in between.

Hughes: And you need both.

Riggs: Yes, you need both, you do. Like every discipline, science or otherwise, there are various ways to get things done, and you generally need all skills.

Hughes:

When Dave and Herb—or maybe it was Dave and Dennis {Kleid} on a later trip--were in your laboratory, there was quite a bit of chaos. Can you remember how it was to bring in these outsiders?

Riggs: [laughs] Well, I actually remember it fondly. Yes, they were working day and night, and Louise was helping them. Let's see, I guess she was pregnant. Yu-Mei Lu I think was also there and sort of took her place. But anyway, sure, they had to deal with these whirlwind, night-and-day experiments.

Hughes: Probably leaving great messes.

Riggs: You know, I don't remember that. But sure, there could well have been. What I remember was that they were getting results, and they were generating data, and I was spending a lot of time with them—sort of data analysis and thinking about what to do next and so forth. I thought it was a great time.

Hughes: Yes, I bet it was stimulating.

Riggs: It was.

Hughes: What about the conceptual genesis of this experiment? Because it necessarily had to be a little bit different, just because of the way the natural gene is constructed.

Riggs: Yes, right. The insulin project required quite a bit of additional protein work—protein purification assembly work—and that wound up being done here in my lab, and that was actually Louise Shively's and my job. So we developed the method for joining the chains, and I researched the literature. It was probably good that I was not an expert in insulin work, because I ran across this published procedure by [Panyotis G.] Katsoyannis, so it's the Katsoyannis procedure. He had—I'll call it the bisulfite method or sulfonation method for stabilizing and then at the appropriate time allowing the chains to join. So there was a published method that could be used to join the A- and the B-chains. So we decided, since

it was easiest for us to make two separate bacterial strains, one producing the A-chain and one producing the B-chain, the Katsoyannis procedure seemed to be the procedure to use. So we decided to use that. By "we" I mean I would discuss it with Herb and Itakura, so this was worked out, as I remember, between the three of us.

Hughes: Was it a laboratory procedure, or would an Eli Lilly producing commercial insulin have used it?

Riggs: I think it eventually was used, until they found—I think they found a better way to do it. But I actually was out of the picture by then.

What we did was to decide to use the Katsoyannis procedure, and then, while Itakura and Crea and other people in his laboratory were making the insulin DNA, Louise Shively and I were working out the joining of the A- and B-chains of insulin. We were able to reproduce the Katsoyannis procedure and get about 10 percent rejoining of the chains, and we thought that was pretty good. We developed the assay, which was the basic—they call it the reconstitution assay—so we could assay for either the A- or the B-chains during purification.

So Louise and I worked out the procedure for joining the chains, worked out the assay for purifying the chains. We also had worked out the cyanogen bromide cleavage method, I guess for the somatostatin project—we didn't talk about that—but it evolved to where Louise and I took on the cyanogen bromide portion of it. So we worked out the treatment of proteins with cyanogen bromide and showed that we could assay for somatostatin. So actually, as it evolved, the radioimmune assays for somatostatin became my part of the project, and then the reconstitution assays for insulin. Okay.

A couple of points I'd like to make. The assays that we had for insulin were not nearly as good. They were an order of magnitude worse than those for somatostatin. So to this day, I'm convinced that if we had gone directly for insulin, we would have not been successful. It would have taken us so much longer.

Hughes: You mean skipped somatostatin.

Riggs: Yes. If we had skipped somatostatin, I think we would not have won the race.

Anyway, getting back to joining the chains: so Louise and I did work that out, so we had the assay developed. She, and later Yu-Mei, were busy with that. So we didn't really have anybody to do the recombinant DNA work, and that's where Heyneker and Goeddel came in. So then they did come in; they did help with the purification; they used the cyanogen bromide procedure, and it all worked pretty well.

Hughes: Well, it didn't work very well at one point. You were having, from what I've read, trouble cloning the B-chain.

Riggs: [laughs] Ah, yes. As I recall, I think that was mainly a mistake.

Hughes: Oh, there was some reversal, wasn't there?

Riggs: Yes, I think that was the reversal. I forget the exact sequence, but you know, instead of C-T-G, it was G-T-C, so somebody did reverse it. I think that was on the insulin project.

Hughes: Yes, it was. So then Itakura or Crea, whomever, had to go back and resynthesize the nucleotides.

Riggs: Right.

Hughes: That was a delay, wasn't it?

Riggs: Well, yes, it was, but not too long. I think it was just a matter of weeks. I don't remember clearly now, but I do remember that it was a very serious and very embarrassing mistake, but understandable—definitely understandable.

Hughes: We haven't talked about Tom Perkins.

Riggs: Ah, yes.

Hughes: Did you meet him in connection with the somatostatin project?

Riggs: I met him once. I went with Herb Boyer and Bob Swanson to Perkins' office, which I remember as being on the top or near the top of one of the skyscrapers in San Francisco. So I did go with them to try to convince Tom Perkins that they [Kleiner & Perkins] should invest in Genentech. So I did meet him there.

Hughes: That wouldn't have been the initial investment, would it?

Riggs: It may have been, because it was quite early on.

Goldsmith: If I can interrupt: based upon the correspondence, Tom Perkins' first appearance in all of this was writing to City of Hope to persuade City of Hope that Perkins and his investors will be behind Genentech, and that Genentech would be a good collaboration partner with City of Hope. So in the first instance, Perkins was working with Genentech to persuade City of Hope that we should participate in the collaboration.

Hughes: I see. Do you remember when that was?

Goldsmith: It was in June or July.

Hughes: Of ’76.

Goldsmith: Of ’76, right.

Goldsmith: You may know that Swanson and others came to City of Hope to make a presentation to encourage us to participate in the collaboration, and it was around that time.

Hughes: What was your impression of Perkins? You probably hadn’t met too many venture capitalists at that time, had you? [laughter]

Riggs: Well, that’s true. I just remember him as a nice guy, soft-spoken. But I was very impressed with the view from his office.

Hughes: He should be in the history because the money after all was an important aspect of getting the research started.

Riggs: Very definitely.

Hughes: Apparently, it was during the insulin research that he had a huge, elaborate party at his house in Belvedere [in northern California]. Did you go?

Riggs: I didn’t go to that, no.

Hughes: Well, it apparently came right at the time that you collectively were having problems with the B-chain. Either Goeddel or Heyneker said that they were terribly embarrassed and trying to keep it quiet. It was somehow in Perkins’ mind a party either to celebrate the insulin success, which of course hadn’t come yet, or, I think probably more likely, to bring in other investors. But in any case, you scientists didn’t want to have it known that this research was not working. [laughter]

Goldsmith: You’re aware, I’m sure, that Swanson came from Perkins’ firm.

Hughes: Oh, yes.

Riggs: The second time I met Tom Perkins, I think it was after the somatostatin success. He came down here, and we had a dinner, very nice dinner, somewhere over in Santa Monica, I think it was. I think that’s about the only other time I met him, just twice.

Hughes: What were your impressions of Swanson?

Riggs: Oh, I liked him. I thought he was a nice young man. [laughs]

Hughes: He was very young!

Riggs: Yes. Let's see, he was twenty-eight, wasn't he?

Hughes: Yes, he was.

Riggs: I was probably already thirty-eight or something like that, so he was ten years younger than I was. Well, I just became very impressed with his ability as a businessman. He was very intelligent, and knew just enough chemistry to ask questions that were kind of annoying. [laughter] But really, that's a compliment, so he did know enough to ask questions and such. Of course, I feel that I was very fortunate to be able to collaborate with—gosh, I was going to start with three, but it's really much more. I was very fortunate to be able to collaborate with a large number of incredibly good scientists and businessmen, but I'll include Swanson in both. He was a scientist and a businessman. He did have training in chemistry, so he didn't know his molecular biology, but he learned. He was just an incredibly talented businessman. Genentech's existence is because of Bob Swanson and his drive, vision, talents.

I guess I might as well say, the scientists that I had the pleasure to work with, Boyer is just absolutely first-rate. Heyneker, Goeddel—Goeddel is just the best of his kind that I've ever had to interact with. Keiichi Itakura—and I could go on. It's just incredible.

Hughes: Swanson, of course, was monomaniacally focused on the success of Genentech. But what about Herb? Herb Boyer still had his lab; he was still a professor of microbiology, and soon to become a member of the biochemistry department. What were your impressions of how involved he was with Genentech and its future?

Riggs: Well, first of all, I do think that he was, I'd say, in sync with Bob Swanson in that they both wanted to establish a company, and I guess Swanson wanted a fully integrated company or whatever it was.

Hughes: Yes, a FIPCO, fully integrated pharmaceutical company. Swanson apparently had that idea from very early on. Do you remember hearing him use that term?

Riggs: Yes. Swanson wanted to build Genentech, and he had a vision how to do that. I think Herb did buy into that and become enthusiastic about it. But I actually had a different vision. It was one that didn't quite work out—well, it did, actually. I saw Genentech as a source of contracts. So I was enthusiastic about the work that Genentech would do, would be contracted out to City of Hope, to UC San Francisco, to Stanford. So I had this vision of a for-profit entity contracting with the nonprofit institutes. And you know, to a certain extent, that does happen, and it has happened. But I think that Swanson was much more correct in his thinking, that in order to really be successful, you needed to do your in-house

research and establish a fully integrated company. So I argued against that—not argued; in discussions, I chose to support research in nonprofit academic institutions.

Hughes: And Swanson would argue back with the FIPCO idea?

Riggs: Yes, he would say, "No, that won't work, we need to establish our own—"

Hughes: Where do you think the idea of a fully integrated company had come from?

Riggs: Where it came from? I would say from his business training. He had enough training, and he had analyzed companies as an investment banker, which I think he worked as for a while.

Hughes: Yes, he did.

Riggs: He was deciding on what companies a bank or an investment firm should invest in. So he had a lot of experience in how companies did run, the ones that were successful, ones that were not, so I think he formulated his plan based on his experience.

Hughes: There was a press conference for insulin. There was also one for somatostatin?

Riggs: One for somatostatin and one for insulin. I'm sure there was.

Hughes: Okay. Well, tell me what your memories are of the press conference for insulin.

Riggs: Hmm. [pause]

Hughes: It was a slightly different group of people because Dave Goeddel and Dennis Kleid were there. Of course, they weren't even around when the somatostatin work was being done.

Riggs: Right.

Hughes: Do you remember anything about the timing of the press conference in respect to publication of the paper?

Riggs: For insulin?

Hughes: Yes, for insulin.

Riggs: I think it was similar—we waited for acceptance. So the paper was accepted in *PNAS* [*Proceedings of the National Academy of Sciences*] before we had any press conference. I think it may have been for insulin that Itakura gave a talk at UCLA. I think it was, actually. Then we had the press conference, and yes, it

was cut short. I think Rachmiel Levine, being an expert in insulin, very appropriately started it off. And then, let's see, what happened. I don't think I wound up talking. I don't remember any more. Maybe because I was so nervous. [laughter]

Hughes: Was there a lot of press there?

Riggs: Yes, I remember a lot of press. At that time, I was very uncomfortable in any such events. If there are cameras or whatever, I definitely was in a nervous state—hyperactivity state. I don't really remember too much about it.

Hughes: The insulin paper was published, as you say, in *PNAS*, but the somatostatin had been published in *Science*. Why *Science* for the first one, which I'm assuming has a much wider audience? And why the *PNAS* for insulin?

Riggs: I'm not sure about the audience. *PNAS* was read by all the molecular biologists, so the *Proceedings of the National Academy of Sciences* is a very good journal. But you know, I think the real reason is that Ernie Beutler became a member of the National Academy of Sciences at about that time, so it may be that he was not a member earlier. He was a scientist here at the City of Hope, and it was convenient. To publish in the *Proceedings of the National Academy of Sciences*, [the paper] has to be of interest to a wide audience and has to be submitted by a member of the National Academy of Sciences. So we had the opportunity to do it, so we did it. I think it was mainly for convenience.

Hughes: As I remember, with the three Cohen-Boyer papers, they were communicated by a member.

Riggs: For *PNAS*, they had to be. At that time, [a paper] had to be submitted by a member.

Hughes: Yes, here it says, "Communicated by Ernest Beutler, October 3, 1978." And then it was published in January, 1979. So it was more convenience than anything else.

Riggs: Yes.

Hughes: I think of *Science* as reaching a wider audience, but you're saying, yes, but the molecular biologists were reading *PNAS*.

Riggs: It was well-respected, one of the top-tier journals in biology.

[End Tape 5, Side B] ##

Interview 3, January 14, 2005

[Begin Tape 6, Side A] ##

Hughes: We were talking about the work on human insulin yesterday, and I understand there was some contention about authorship. One of the results was that there were two papers published on the work. Do you remember?

Riggs: I don't really remember much contention. I thought it was very logical, and I was fine with dividing it into the two papers because at that time the DNA synthesis methodology was a very large, novel aspect of what we were doing. So that gave Itakura and his chemists the opportunity to get some recognition—that was nice. So I liked that idea. But what I would comment on is, I think everyone on the team would have been very happy to have Herb Boyer's name be on the paper. He didn't do any work on the details of the project, but he certainly helped get it started. The whole process was part of the original plan. I don't know why, and I think this is correct, I don't know why he decided not to be an author. I can speculate that it had more to do with his situation and some of the criticism that he was receiving up at UC San Francisco. But I'm really just speculating, and I think only Herb knows. I think only Herb Boyer knows.

Hughes: Yes, that was certainly a period when he was very much under siege, particularly from colleagues in his own department. I think it was a very painful period for him.

Riggs: Yes. I would just repeat that as I remember it, I think everyone on the insulin team would have been okay with him being an author.

Hughes: That's an interesting point, because the early people at Genentech make quite a thing of how Herb let them be authors without having his own name on papers. I'm sure there was some goodwill, maybe largely goodwill, but the criticism could have been a factor as well.

Riggs: Yes, I agree with you. As a matter of fact, one of the decisions or ideas that Herb Boyer was a champion of was that the scientists at Genentech be allowed to publish, the only restriction being that if patents were going to be issued, they needed to get those patents in before publishing. So he was a strong advocate for publishing and a strong advocate for not

micromanaging. So he did let the scientists make their own decisions. Now, I'm sure there was at the board level or at the management level a lot of guidance, and I do think that he was very active in many aspects of the management of Genentech, from the early days. But yes, I do think one of his decisions was that he did not need to be on every paper. So just the opposite of the German system, where the professor is on every paper, regardless.

Hughes: I wonder how often he was even physically at Genentech, once there were facilities to be at. [laughs]

Riggs: I don't know. I was down here [at the City of Hope] most of the time, so I don't know.

Hughes: I get the impression, and it would go along with what you're saying, that he certainly wasn't a constant presence. After all, he still had his professorial duties. He had a very active lab, and he had enough going on right at UCSF. So just from that standpoint, I would think it would have been difficult to really have been a presence at Genentech on anything approaching a daily basis. And I don't think he was.

Riggs: You know, I really don't know one way or another.

I did spend some time at Genentech in the summer of 1980. I did see Herb Boyer, but just a few times. But again, I really don't know how much time he was spending [at Genentech].

Hughes: What were you doing in 1980?

Riggs: That was the antibody project.

Hughes: That was the antibody project, which we'll get into in just a minute.

There were two previous visits to Genentech, right? Or more than that?

Riggs: Certainly more than that.

Hughes: Did you observe a different lab culture than you were used to here?

Riggs: [laughs] Not dramatically different. But yes, different, let me say, maybe in the level of A, hard work, and B, they also played a lot. They were playing Nerf ball—I mean, they were young. They were all in their, I don't know what, early thirties probably. Herb Heyneker, David Goeddel, and even Tom Kiley, and others whose names I'm forgetting right this second were quite a dynamic and social, interactive group.

Hughes: They were all roughly the same age, weren't they?

Riggs: Roughly. I guess I don't really know their exact ages.

Hughes: I'm suspecting that Herb Boyer was the oldest.

Riggs: Yes, he was the oldest. I think he's a couple of years older than I am. [laughs] So we were the old men.

Hughes: The old men at thirty-five, or whatever you were.

Riggs: [laughs] Right.

Hughes: Well, do you have anything more to observe about human insulin?

Riggs: I think we've pretty well covered that. Nothing comes to mind right now.

Hughes: Was it a smooth transit to the human growth hormone project? Had that from the start been a goal, to clone and express growth hormone?

Riggs: It was a goal early on. I think it would have been a decision made by Boyer and Swanson. I don't remember when that decision was made to go for growth hormone as the next attempt by Genentech. But I do remember it was early on, and so just as soon as the insulin was—when the DNA synthesis cloning work was done, I know there was a team that they started at Genentech to work on the growth hormone project. Growth hormone was a very significant project which I was not involved in. As I mentioned when we were talking yesterday, I wanted to do work on, I'll call it vaccines, using recombinant DNA for vaccines, and also antibodies. So I was not significantly involved in the growth hormone project. But it was a very important project. That was where they combined the cDNA [complementary DNA] approach with the chemical DNA synthesis. I think that was where they were able to show they could get expression without a precursor peptide. So that was an important project, and Itakura—

Hughes: He did the synthesis, didn't he?

Riggs: —was a key person for that project. But I wasn't.

Hughes: I asked him yesterday, or it came out anyway, why the whole molecule wasn't synthesized. I know it's much larger than insulin or somatostatin. Dr. Itakura acknowledged that, but he said he could have synthesized it.

Riggs: Yes, I think he's right. I guess when you add up all the man-years needed, the chemical synthesis might have even been approaching being the most effective. But I don't think that was the general opinion. Most thought for larger proteins that the cDNA approach—isolating the messenger RNA, using reverse transcriptase, and getting most of it—and then using the chemically synthesized DNA just for editing purposes—you might say editing and positioning, sort of

the finishing touches that were crucial. But these days, I'm sure you could, if you had the sequence, which we do now know, that just to make growth hormone—gosh, I don't remember now what size it is—but I suspect it could be made in just a day or so.

Hughes: I don't know the number of nucleotides, but I read somewhere that it was roughly four times the size of insulin.

Riggs: Okay.

Goldsmith: I think Keiichi said, what, 190 amino acids?

Riggs: So that would be 600 [nucleotides]. Yes, you could probably—I don't have to qualify it—sure, you could make the DNA fragments in probably less than a couple of days. These days, the whole synthesis of the growth hormone gene would probably take you maybe a week or two.

Hughes: Was the generalizable aspect of this work the fact that you could combine the cDNA approach and the DNA synthetic part?

Riggs: Sure.

Hughes: Had anybody else done that?

Riggs: No. As I recall, that's the first time that was done. As I mentioned earlier, the other real importance there was that I think they got it synthesized without a precursor peptide. That was important too.

Hughes: And the reason that DNA synthesis had to be included was because [Peter] Seeburg didn't have the whole natural gene.

Riggs: That's correct, although I do not know whether or not Seeburg's gene was ever good.

Hughes: Why was that? Or is that just a technicality?

Riggs: I would say just a technicality. In those days, it was hard to keep ribonuclease from chopping the RNA a little bit, and then also, the reverse transcriptase might not make it all the way to the end. So, yes, it was just a technical detail. It made it hard to get the N-terminus of the gene for growth hormone.

Hughes: I know you were watching from the sidelines, so to speak, but as far as you know was the interaction between the two teams similar to what had occurred in human insulin and somatostatin? The way the Genentech scientists and the City of Hope scientists collaborated?

Riggs: Well, they were certainly collaborating—

Hughes: There was nothing really new in the relationship?

Riggs: No, I don't think so. Roberto Crea went up to Genentech fairly soon, but I think that was probably after the growth hormone. I don't remember.

Hughes: No, he was there by September, 1978, which is before Seeburg came over. Seeburg was still at UCSF. Why was Itakura nonetheless in charge of the synthesis when, by the time of the growth hormone project, Genentech had its own on-site DNA synthesis capability?

Riggs: Right. Well, of course I don't really know, but clearly, they were in a transition, so it was in that transition period. I'm sure planning began--I would speculate that planning began before Roberto Crea decided to go to Genentech, and then that whole thing was sort of in a transition period. I think that would be the answer.

Hughes: We've talked about how somatostatin was largely a demonstration project, without any real hope of having somatostatin become a marketable therapeutic product. The way I am imagining human growth hormone was initially conceived was that it was going to be used for individuals of short stature. And that's not a very large population, right? So why was human growth hormone chosen as the next step after human insulin? Human insulin was a so obviously highly marketable and a needed innovation.

Riggs: Well, of course, I really don't know. I don't remember very much. But I do remember that Swanson somehow convinced himself that there was an adequate market for growth hormone.

Hughes: I wonder when he knew that orphan drug status was a possibility for human growth hormone.

Riggs: Again, I don't know. It's certainly a possibility.

Hughes: Do you have something to say?

Goldsmith: Yes. I think Swanson turned out to be right, because there actually was a sizeable market.

Hughes: Oh, I know that. But Swanson didn't know that at the time.

Goldsmith: Do we know that?

Hughes: Well, we don't know that.

Goldsmith: Right. They entered into a license agreement with KabiGen very early, for growth hormone. And [Eli] Lilly subsequently went into the growth hormone business as well. So I think there must have been, or was likely to have been some knowledge.

And just one correction—I'm sorry—on the somatostatin: I think Art said before that, while they didn't think it was likely, I think Art went into this thinking that there was a possibility that somatostatin might have some practical applications.

Riggs: There was a possibility, yes. Sure.

Hughes: I know that was a thought. But it certainly wasn't as clear as it was with human insulin.

Goldsmith: Right, it wasn't obvious.

Hughes: As I remember, in the growth hormone paper, its uses other than just for short stature are mentioned, which I thought was kind of amazing. And of course, now we know that yes, it has been used for other conditions.

Riggs: I'm not really remembering clearly, but yes, I think there were other possible uses for it. I remember reading at about that time there was a degenerative brain disease caused by a—I can't pronounce it—prion, and the quality control for the growth hormone derived from pituitaries became a real issue.

Hughes: That's right.

Riggs: And that may have been happening at about that time.

Hughes: It did, and it speeded up the FDA approval, because the FDA had been stalling on approving Genentech's growth hormone.

Riggs: But like I say, I wasn't really part of that project, and so there's no real inside information there.

Hughes: The research turned out to be very controversial for other reasons. There were a lot of legal repercussions.

Riggs: Yes, that's right.

Hughes: You're probably pretty glad you hadn't been part of it.

Riggs: Yes. I met Peter Seeburg once, and that was after he was at Genentech. But that's the extent of my interaction with Peter.

Goldsmith: At the time, did you know that growth hormone was going to be a fountain of youth?

Riggs: [laughs] That's right—that may have been already happening, the hype about that.

Goldsmith: Because I view it as a fairly recent phenomenon.

Hughes: Yes. That's why I was interested in the fact that that paper, published some time in the late seventies, was already speculating about other uses for growth hormone.

Goldsmith: It's hard to believe that Art is ninety-two. [laughter]

Hughes: Here is the paper. It was a 1979 paper, and it mentions the possibility of using growth hormone to treat bone fractures, skin burns, and bleeding ulcers. So obviously, Genentech already had a vision for a market larger than replacing pituitary growth hormone.

Riggs: That makes sense to me, yes. I'm sure that's true.

Hughes: One of the obvious differences between science in academia and science in industry is that in most companies—perhaps subtracting a small fraction of really basic, open-ended research firms—marketability has to be a consideration.

Riggs: Oh, definitely. Swanson was correctly focused on that. He knew that he had to have something for which there was a market, which would mean there would be some use.

Hughes: One that springs to mind is thymosin. Genentech cloned the gene for thymosin, but then the project was killed because its therapeutic use was very doubtful—which must have taken some adjustment for scientists who in those days almost exclusively had come from academia. They'd had—in their sense—a successful outcome, but the business heads in the company said, you might have a scientific success, but it's not a business success.

Riggs: I don't know if I ever knew about thymosin. But I do know that other products, yes, the scientists did have trouble [laughs] if and when the management killed some of their pet research projects. Oh, yes, it definitely was something they had to come to grips with. And I think many of them got upset about it.

Hughes: You probably would have too, would you not?

Riggs: Oh, yes. [laughter] Absolutely.

Hughes: Art, did it ever cross your mind to form or join a company?

Riggs: Not seriously. Of course it crossed my mind, yes. Did I think about it? Yes. But my goal became to do what I did do for a number of years, to continue to work with Genentech, but just do contract-type work with them, because what they wanted to do was really exciting, state-of-the-art research at that time. And I thought that it would become a source of funding for my laboratory and others. So that was the path that I decided to take. I didn't have any difficulty making that decision, to stay in what you might say is more basic research, academic-style research.

Goldsmith: Sorry—I guess when you talk about contract work, you're talking about doing these kind of collaborations like you had been doing?

Riggs: Yes, absolutely.

Hughes: So in a sense, you had the best of both worlds, didn't you?

Riggs: Oh, yes. I really enjoyed all of the collaborative efforts with Genentech. Herb Heyneker was great to work with.

Hughes: What about sitting on corporate boards? Have you done any of that?

Riggs: No, I haven't.

Hughes: The same sort of reason?

Riggs: Yes. I really don't want to. I did get into management and became the chair of the biology division, and that was about 1982. Between trying to keep my own research going and funded by NIH— I kept writing these NIH grants, and between you might say administrative duties and the research, I didn't feel that I had the energy—that's the right word. So I don't think I had the energy—

Hughes: To do it all.

Riggs: To do it all, yes.

Hughes: All right: antibodies. How did that project evolve?

Riggs: I think I've already mentioned that after we were successful with somatostatin, I definitely started thinking about how we could apply this technology, and what was the most important thing to do with it. I think it was probably because of my background in Melvin Cohn's laboratory, and then because I was actually interacting with Charlie Todd's laboratory. They were immunologists, so it was reasonable. I was reasonably knowledgeable about immunology, so I thought the most important thing was to use the precursor peptides and select them to be

antigenic. I actually knew that beta-galactosidase was a very good antigen, so it did stimulate the immune system and was nicely recognized as foreign by mammals. So my idea was to use the precursor peptide to stimulate the immune response and then add on the specific target protein. So I did start working on that, and that was actually the first project on immunology that I started. I did get funded. Funding for that project was from Genentech, and we tried to clone a gene from Coxsackie virus, which I thought might be one of the causes of diabetes. Even though that hasn't been really established, it's still, I think, a possibility. The general idea is that an infection, virus infection or whatever, triggers an autoimmune response, causing diabetes.

[End Tape 6, Side A] ##
[Begin Tape 6, Side B]

Riggs: We did try for a year or so to clone the coat protein of Coxsackie virus. But the person working on the project was not successful.

Hughes: Is that a large virus?

Riggs: You know, I forget now what size it was. Not huge. It was thought to be doable.

Hughes: And there was evidence in the literature that there might be a connection with diabetes?

Riggs: There was, yes. Not a proven connection, but just speculation, hypothesis. So one hypothesis was that the Coxsackie viruses were a major cause of juvenile-type diabetes. But we didn't succeed in cloning the gene for the viral protein. So I thought I would just go for cloning antibodies, and among other things, converting a mouse antibody to a human. Making what's now called the chimeric antibodies. So I decided to switch the work that was supported by Genentech from that project to cloning recombinant antibodies. As I remember it, somehow I started talking to people at Genentech, and Herb Heyneker was interested. So he and I then started discussing the cloning of recombinant antibodies and decided to go for it. As I recall, there was one other person that I should mention. The opportunity to recruit him from Israel also stimulated this whole project, and that was Shmuel Cabilly. Shmuel Cabilly got his Ph.D. in immunology—so he was an immunologist, and he wanted to come to the United States and come to my laboratory.

Hughes: He had applied to you? Is that how you knew of him?

Riggs: He applied, yes. So he applied to me, asking if I had a spot for him in my laboratory, and I said, Oh, yeah, this guy's got an immunology background. It turns out that he was interested in the recombinant antibody project, so he came and was to be a postdoctoral fellow in my laboratory, and then we were going to collaborate with Herb Heyneker at Genentech on this project. So that developed

into a very nice collaboration, and we were able to clone the antibody genes and get some patents that turned out to be quite valuable.

Hughes: Who was doing what?

Riggs: Shmuel started the project, and he started by, oh, say, working on the reconstitution of antibodies. So he did some work on that.

Hughes: Meaning, making the light and heavy chains?

Riggs: When you make antibodies, they're also a two-chain, rather complicated, and considerably larger protein. So he worked on putting heavy and light chains together and et cetera, and also on some model—he had sort of a model system experiment. But he also then went to Genentech and sort of learned to become a molecular biologist from Herb Heyneker.

Hughes: How to clone, essentially?

Riggs: Yes. So Shmuel sort of shuttled back and forth between my lab and Genentech. And like I say, his background was immunology, so he was learning to be a molecular biologist during this period, and he learned pretty quickly, and he was a very creative guy.

Hughes: The significance, I gather, or one of the significances, of this work was that up until then antibodies had been exclusively or largely obtained from hybridomas?

Riggs: Yes.

Hughes: Maybe you could explain the advantage of being able to derive them from *E. coli*?

Riggs: Well, it wasn't just *E. coli*, but it was using recombinant techniques to change them. Actually, I remember, there was some immunologist at Genentech who was not very enthusiastic about the project, and I forget his name right now. He would say, "Why bother doing this?" Because by about 1980 or so, there were claims that one could get human hybridomas, so you could get human proteins just by using this hybridoma technology. So that's the way to go [he thought]. But I never agreed with that, and it was because when you get the recombinant DNA approaches perfected, then you don't have to stay with what nature provides you. You can make changes.

Hughes: Yes, you can manipulate—

Riggs: You can make your own changes. So it's the manipulation and the changes and the making of these novel, unnatural, improved antibodies that was the exciting part, that was the exciting potential of what we were trying to do. And we very

much knew that we could, that when you put a mouse or a rabbit or any foreign antibody into humans, they make antibodies against what's called the constant region. Most of the immune response is against what's called the constant region, and that's the region that's the same in all antibodies. There's the variable region and the constant region. I knew that most of the immune response was directed against the constant region. So the idea from day one that we started that project was that once we had it, then we could just take the variable or the specificity that we got from the mouse hybridoma, and then we could tack on the human constant region, and we'd have an antibody molecule that would be relatively non-immunogenic, and you'd have the desired specificity. It turned out that it's true, so all that was true.

Hughes: You probably had been following the earlier efforts, beginning I think with Hybritech. I think that was the first company founded on the basis of monoclonals.

Riggs: Yes, and hybridomas, right.

Hughes: Yes, and hybridomas. It was founded in '78, '79, something like that, a very early company. My very superficial knowledge is that the problem was that they were not humanized antibodies—they were mouse antibodies?

Riggs: Yes, all of the hybridomas at that time were all mouse. Right.

Hughes: I think I got this from talking to Herb Heyneker: the hope was to move as rapidly as possible—but it actually wasn't that rapid—from an animal antibody to a human antibody. So what you were doing was maybe a halfway step? Because you could get the antibodies to be partially humanized?

Riggs: Well, the chimeric antibodies, using the terminology now—I actually would dispute the terminology as it's used now. The idea was to convert, to change the mouse antibody so that it no longer stimulated immune response in the human. To me, that's the humanization—

Hughes: Meaning that you don't have to have an entirely human antibody, as long as it doesn't stimulate a bad reaction.

Riggs: That's right, and we already knew that there were the hyper-variable regions. There's the variable regions, but then within the variable region of the antibody, there are hyper-variable regions. We already knew that most of the specificity was generated by the hyper-variable region. So we knew the hyper-variable regions were the most important. So in theory or on paper, one could use the hyper-variable regions for specificity and change all the rest, either to be perfectly human or to be more nearly human.

Hughes: And you did that?

Riggs: We had the concept of what I would call humanizing antibodies when we started the project. We were able to specify how to make a chimeric antibody, so we were able to do a solid specification of one way to accomplish that. I think everything that's being done these days is just applying the same thing—well, I'd say, just continuing to refine what we did.

Hughes: Meaning making more and more of the molecule like the human.

Riggs: Right.

Hughes: Did you carry it far enough so that you were able to overcome the allergic problem?

Riggs: We never tested it, but later on, when chimeric antibodies were very thoroughly tested, Herceptin for example, they turned out to be reasonably non-immunogenic. An antibody can never be totally invisible to the immune system because you get antibodies against the specificity-determining sites. So you actually do get that—but they tend to be minor.

Hughes: Why would nature do that?

Riggs: Oh, it's just the way the system works. It tries all possibilities, and if anything binds, then that stimulates the cell to grow—

Hughes: To react.

Riggs: So you can never get away from it completely.

Hughes: Because you've got to maintain those highly variable areas.

Riggs: Yes.

Hughes: There's nothing you can do about that.

Riggs: Yes.

Hughes: So again, it's this ability to manipulate and modify—it's sort of a theme, isn't it.

Riggs: Yes.

Hughes: Why did you use CEA, carcinoembryonic antigen? That was just the usual substrate for this work?

Riggs: No, that was due to my friends here at the City of Hope, and I think I've already mentioned Charlie Todd a couple of times.

Hughes: Yes.

Riggs: He was one of the world's authorities on carcinoembryonic antigen, which is almost always abbreviated CEA, so we just say CEA. That's a protein made on the surface of many cancer cells. Colon cancer and breast cancer, for example, very frequently express this CEA antigen. The cancer cells do but the normal cells do not. So most normal cells don't make any CEA, but cancer cells do. So this was a very nice target. It was the magic-bullet concept. We thought that having an antibody against CEA would allow the antibody to recognize, seek out, bind to, become concentrated in the area of the tumor. Then if it didn't kill directly, it could be used to carry in some toxic agent such as a radioactive element. That's why we used CEA, because I had an expert next door who had a postdoctoral fellow named Jack Shively whose wife worked in my lab. So for me, CEA was the logical choice.

I forgot to mention one other thing: in Charlie Todd's laboratory, and I think Jack Shively was involved, they had a hybridoma. So they had the hybridoma that we could use as a starting point. To date, the use of anti-CEA antibodies has not yet been widely used. But they will be. The City of Hope, and others—so we're not the only ones now—are still doing experiments using recombinant antibodies directed against CEA. I haven't been involved in that project for a long time. But in the late eighties, Jack Shively and I continued to work with recombinant anti-CEA antibodies, and that work was funded by the National Institutes of Health. That project has grown steadily since then. So there's quite a few clinical trials underway now here at the City of Hope and elsewhere, using anti-CEA antibody.

Hughes: Are there any preliminary results?

Riggs: The imaging is working very well. So as a way of detecting small metastatic tumor clusters, yes, that's working pretty well. Probably the best answer is not yet, but I expect that it will.

Hughes: Potentially, it would work against all cancers because they all have CEA?

Riggs: Not all cancers have CEA, but I think it's something like 60 percent of breast cancers do display CEA and probably a higher percentage of colon cancer does.

Hughes: My understanding is that the usual approach is to try to find a way of discriminating, so that your drug or radiation or whatever is going to target only the cancerous cells. Well, this seems such an obvious—

Riggs: Well, I can give you a general answer. It's taken time, for the same reason that almost every other target has taken a long time. Rituxan was actually the first major success, and when was that, five years ago?

Hughes: Oh, something like that.

Riggs: Actually, I know a little bit more about Herceptin. Work on Herceptin really started in the eighties.

Hughes: At Genentech?

Riggs: By the late eighties at Genentech, yes. But one didn't start treating a large number of patients until at least ten years later. We can come back to CEA: where the work on that is now is that you have to show that it's safe, so you have to do safety tests. Then more specifically for CEA, like most projects, you run into certain problems. The problem with CEA is that the tumor is making the CEA, but enough of it is shed—maybe clipped off by protease, whatever—that it winds up in the liver. So the antibody also goes to the liver. The effort has been to reduce the take-up in the liver and also kidney. The kidney tries to excrete it. It's really been a great protein engineering effort that's been going on here. They've been trying, by playing with the molecule, to decrease—the kidney is actually the one that's the more sensitive to radiation—the exposure to the kidney and increase the exposure to the tumor. That work is going very well. The clinical trials have shown that it's not toxic, so they can do it without any great toxicity. So that's where the work here is.

Hughes: Turning to what people call the Cabilly patents, you and Cabilly are inventors?

Riggs: Yes.

Hughes: Sole inventors?

Riggs: No. Herb Heyneker and a couple of others from Genentech.

Hughes: Who are considered to have had a conceptual part in the invention?

Riggs: That's right, yes.

Hughes: Would you consider the patents as broad for antibody production as, for example, the Riggs-Itakura patents are for the synthetic recombinant DNA approach?

Goldsmith: You know what: let's go off tape. [Goldsmith halts discussion of Cabilly patents.] [tape interruption]

Hughes: Do you consider this work the basis for the antibody-based drugs that Genentech and other companies are now producing?

Riggs: Yes, it certainly paved the way and was the first of its kind, and it definitely was an enabling technology that became very important, no question about it.

Hughes: Anything more you want to say about the antibody work?

Riggs: I think we've pretty much covered it. [tape interruption]

Hughes: There were two things, both of them happening in 1980, which had an impact on intellectual property protection in biotechnology. One of them was the Supreme Court *Chakrabarty* case, which, to put it simply, said that living organisms or, as they called them, life forms, were potentially patentable. They were not excluded from the right to patent. The other one was the Bayh-Dole Act, also of 1980, which made it possible for universities to patent research that had been federally funded. Were you paying attention to these developments, and if so, what did you think, and did it have an impact on what you were doing?

Riggs: I was aware of the *Chakrabarty* case. But I don't think I was really focused in on it, you might say, because part of what we did was methodology. So some of the patents were methods patents. I didn't know how significant it would be, being able to patent a particular construct, that became possible after the *Chakrabarty* decision. It certainly turned out to be a very positive decision for the biotechnology industry, without a doubt. But like I say, I don't think I really focused in or worried about it too much.

Did I even know about the Bayh-Dole Act at the time? I probably did, but right now, I can't remember that much about it.

Hughes: Were you aware, though, that federally funded research previously could not be patented?

Riggs: That's what I was trying to remember. I don't think—

Hughes: Or that the federal government held the patents?

Riggs: That's right, they did. I don't remember what I knew then. [tape interruption] But I didn't do any work covered by NIH for the Genentech project.

Hughes: Right, it was all Genentech money, wasn't it?

Riggs: Yes, it was all Genentech money, and so I did know that either City of Hope or Genentech would own it.

Hughes: Yes. So it wasn't even an issue.

Riggs: No, it wasn't an issue for me, not at the time. Then by 1980, again, I wasn't doing any work that had been previously supported by NIH, so it was not an issue that I had to worry about.

Hughes: Yes, that makes perfect sense. Is it a legitimate question to ask if you were contributing to the writing of the patent applications today, would you write them differently?

Riggs: Well, first of all, the patent lawyer is the one that writes them.

Hughes: I know that, but I meant in the sense of talking with the attorney. You had to discuss with the attorney the science that went into the application.

Riggs: Sure, that's right. First of all, I hadn't thought about it, so it's a new idea. I don't know that I would change anything.

[End Tape 6, Side B] ##
[Begin Tape 7, Side A]

Hughes: How did you feel at the time about the accelerating commercialization of biology? As represented by the number of patents coming out and the number of people going into industry, consulting for industry, or sitting on corporate boards?

Riggs: My thinking hasn't changed very much. I was an advocate for [patenting], maybe in part because I did come from a chemistry background, so I knew that there was already that opportunity for chemists. So to see that also become possible for biologists and molecular biologists—I was okay with it.

One of the results of the somatostatin project for me was that I came to understand the tremendous value to basic research, research in general, that patents have. I became aware that one of Swanson's great decisions was to allow our team to publish. I want to give credit to Boyer, too. So Boyer and Swanson. Or let's say Boyer was able to convince Swanson—

Hughes: Convince Swanson—that's really the way it went.

Riggs: That's right. And of course, I was arguing the same way too, that we had to publish. Matter of fact, I would not have accepted the contract, I would have not done the work, if we didn't have the right to publish. So the way it was able to go forward was that we had to submit the patents, and then we could publish. I became aware that the alternative was secrecy. So trade secrets was the alternative. So either you patent and publish, and you try to tell the world about it as much as you can, or the alternative is you keep it a trade secret and you don't tell anybody about it. I came to understand, and I still think it's definitely true, that patents are wonderful because they promote the flow of information for research. So the research scientists, even though there's a patent, could continue working on it. That's pretty much still true today.

Hughes: [tape interruption] Your main scientific interest nowadays is in epigenetics?

Riggs: Yes. [laughs] Well, the work in my laboratory. I am interested in a lot of work that goes on around the country and in siRNA work especially.

Hughes: Maybe first define what you mean by epigenetics?

Riggs: I will. My own work is mostly on epigenetics. Epigenetics is a word that was first used back in the thirties, and it was used in the context of development of an embryo into a mature organism and developmental decisions in that context. It fell out of use. It was not commonly used, but then I, among others, began using it with the following meaning. I'll start by talking about genetics and mutations. [phone rings] [tape interruption]

So, in order to explain epigenetics and epigenomes, I need to talk first about classical genetics. So we all know that a gene has four nucleotides: A, T, G, and C. It's the order of those nucleotides that makes a difference. Occasionally, you can get a mutation, and that's one where the sequence, or the letters of the word, actually change, so you actually substitute one letter for another. That changes the meaning.

Well, first of all, the idea that there are only four nucleotide bases in DNA is an oversimplification. There are actually five. Even at the time that Watson and Crick did their work, they knew that about 4 percent of the cytosines, of the C's, were actually a different molecule, a 5-methyl cytosine. At the time, they ignored that 4 percent, and they were correct in doing so. But it turns out that methylated C, 5-methyl cytosine, does change the information content, or let's say it's an important component of the information contained in the DNA, on the DNA double helix. So nobody knew what the function of 5-methyl cytosine was, and especially they didn't know the function in mammals. Now, I've described a little bit of genetics, what a mutation is. Of course, once you get the change in the nucleotide sequence, once you've changed a letter by mutation, then it's inherited. So that change is passed on to all progeny of that cell, and if it's a germ cell, it's passed on to the next generation of animal, be it mouse or human.

Well, epigenetics describes a heritable change—so now it is inherited—but it's not a change in the primary nucleotide sequence. So whatever the sequence of A, T, C, and G is, that does not change. What I proposed in 1975 was that the molecular mechanism of epigenetics was a change in 5-methyl cytosine. So I proposed that 5-methyl cytosine was part of what would now be called an epigenetics system that was important for mammals.

Hughes: What was your basis for pointing your finger at 5-methyl cytosine?

Riggs: [laughs] Before I answer that, let me give you a succinct definition, because I gave you a very long definition of epigenetics. Epigenetics is the study of heritable changes that are not due to changes in the primary base sequence. So

that's the definition that I like, and I was one of the first to start using that definition. So as genetics has to do with heritability, so epigenetics also has to do with heritability. So now back to your question of why did I make that postulate. Well, there's actually a Herb Boyer connection. [laughter]

Hughes: Yet again.

Riggs: I forget, have I already said for the record that I came to the City of Hope to work with Susumu Ohno on X-chromosome inactivation?

Goldsmith: Yes.

Riggs: Okay. Well, I did continue to work on X-chromosome inactivation, and I was interested in this question of how you can have two identical sequences that were yet recognized as being different from one another—that one was totally inactive; the other one was normal, all the genes were normal on that chromosome. So I was puzzling about that connection.

When I started interacting with Boyer, and when I went to Herb Boyer's laboratory early on, '73, to take the beta-galactosidase operon to him, I was exposed to the restriction enzyme information knowledge base. So I started reading about restriction enzymes. It turns out that bacterial and bacteriophage restriction enzymes—well, they're all bacterial restriction enzymes--the bacterial restriction enzymes form 5-methyl cytosine. So 5-methyl cytosine is very important for these restriction enzymes which Herb Boyer was studying.

I won't try to explain in detail, but I learned that the properties of the type 1 restriction enzymes could be used to explain the heritable differentiation of two identical DNA sequences. So I thought about it for a while, and I researched the literature, and then I wrote a paper. You could call it a theoretical paper because there was no original experimentation for it—and that paper was published in 1975.[6] In that paper, I said that X-chromosome inactivation could be explained by 5-methyl cytosine. I also proposed a mechanism called the maintenance methylase mechanism. I predicted the existence of an enzyme that would result in a system that would preserve the differentiated state.

Now, it turns out that those predictions I made are true. So there is an enzyme that preserves the methylation of the 5-methyl cytosine pattern in DNA. Let's say the primary information is indeed encoded by the four nucleotides. But then there's also an enzyme that comes after DNA replication that sort of decorates the DNA and decorates the chromosomes with a 5-methyl cytosine. It makes 5-methyl cytosine, and so you have additional information sort of written on the DNA. The methyl group goes into the major groove—so it's actually

6. A.D. Riggs, "X inactivation, differentiation, and DNA methylation," *Cytogenetics & Cell Genetics* 1975, 14:9-25.

information written in the major groove of the Watson-Crick helix. That information then is passed on to the next generation of cells. So the progeny cells have a memory of the differentiated state of the parent or mother cell, and to a small degree, it can actually be passed on to the next generation. In plants, the epigenetics does actually survive through meiosis, and there's just a few cases in mammals where it also does. It turns out that mammals and vertebrates—but it's actually most used in mammals—mammals require this epigenetic system in order to be viable. Let me come back to that.So I made a number of suggestions, made a number of predictions, but I did not use the word epigenetics in that paper. [laughs] That came later—epigenetics did not become common terminology until, gosh, the '90s. So that paper was published in 1975, and it's one reason, of course, why I never considered switching and going to Genentech. I wanted to stay and work on epigenetics.

So I made that proposal in 1975, and—

Hughes: And that particular statement?

Riggs: Well, I did actually suggest it was very important for differentiation of the X chromosome in mammalian development. So yes, I did predict that 5-methyl cytosine would be important for mammalian development. And that prediction did become true. However, it took time, because after I made that proposal—and it did have effect; it was not ignored by other developmental biologists and molecular biologists—they very soon found out that yeast does not have 5-methyl cytosine. I and a colleague in Israel, Aharon Razin, we found out that *Drosophila* also does not have 5-methyl cytosine. So without exaggeration, most molecular biologists lost interest—

Hughes: Because it didn't seem to be a generalizable phenomenon?

Riggs: That's right. And it wasn't until about 1990, '91, that the technology had reached the point where you can knock out the gene for this enzyme that I predicted. And it turns out when you do knock that out, that gene, it's embryonic lethal.

Hughes: That was pretty good evidence, wasn't it?

Riggs: It's a twenty-five-year story, or thirty-year, by now. But for that and for other reasons, the interest in epigenetics has been steadily increasing, and not linearly. It's been sort of an exponential increase. In 1975 I think there were two papers in the entire literature that talked about DNA methylation. Now, there's thousands every year. And the majority of those are on cancer. One of the mistakes that happens during the progression of cancer from the pre-cancerous state to a fully metastatic state—and of course, it's only the metastatic state that's lethal—so epigenetic changes are now recognized as being as common as mutations. In many cases, not all, but in some cancers, they're the most commonly found changes. They're not real mutations; they're these epi-mutations.

Hughes: Are the mutations occurring to the nucleotide sequence and the ones to this methylation system in some way through many steps connected with what's happening external to the cell?

Riggs: They're part of the readout; yes. The cell, of course, has receptors that gather information from the environment, and then they signal, usually through a complicated pathway, to the nucleus, and then the transcription factors and other proteins involved in gene regulation change as a result of the information from the environment.

Well, the change in methylation of 5-methyl cytosine happens at the DNA level, but how it works is by affecting the interaction [of DNA] with these transcription factors. So 5-methyl cytosine directly and indirectly affects the functioning of transcription factors, yes.

Hughes: One of the criticisms of the Human Genome Project was that by focusing so much attention on the genes themselves—I don't think they were thinking of these epigenetic systems which are so closely associated with the gene— The point as I took it is that you're not explaining enough by just working out what the genetic sequences are. There's much more involved.

Riggs: Right, right.

Hughes: You've known that forever. Well, at least since 1975.

Riggs: That's correct. And I think I can speak to both sides.

Hughes: All right.

Riggs: A, the critics were wrong. They were right, but they were wrong in opposing the Genome Project, because the benefits towards understanding the whole process are just fantastic. But, they were also right, [laughs] they were also right in that the sequence is not enough, and so we need to go back and determine the epigenome. There's even a word for it now. There is an epigenome project that is underway.

I think it would be fun to point out that at the time the Genome Project was getting underway, so this would have been in 1990, 1991, approximately, I had developed, with Gerd Pfeifer here, and also in collaboration with Barbara Wold at Caltech, a method called ligation-mediated PCR. I'm not going to try to explain what that is, but I wrote an application and submitted it for funding from the Genome Project. The application was, we'll automate ligation-mediated PCR, and we'll determine the position of every 5-methyl cytosine in the genome.

Well, that application was premature, [laughs]—

Hughes: In other words, they turned it down?.

Riggs: It's probably an oversimplification to say yes, but I'll say yes. To be fair, the attempt was premature. We did wind up automating the procedure, but just a few years ago. There's other techniques that have been developed that are probably more appropriate for determining methylation pattern in the entire genome. But there is a group primarily in England and Europe that is proposing to do that. They are well along in starting to determine the methylation patterns for the entire genome.

Hughes: Is one of the things to take from what you are saying, to a certain extent, technology has limited revelation of this cooperative system that also results in mutations?

Riggs: Yes, well, one question there was, has technology limited—

Hughes: Yes, has there been a technological aspect, that it was not easy to get at this system?

Riggs: That's correct, absolutely. When I started, there was no way to determine methylation patterns in the DNA. Actually, you may know the name Judy Singer-Sam. We developed one method, but now there's a number of methods to determine it. But I'd like to emphasize that I personally think that technology is limiting, or is the most important part of science. Techniques and technique developments, I personally think, are more important than the hypothesis-driven research. Now, I think I'm in a minority—probably a fairly large minority—but I think I'm in the minority. Most would fully subscribe to the idea that advances in science are made by having a great idea, then developing a hypothesis to test that idea, then doing the work, then reiterating that process. Well, I really think it's very important, but I think let's say half or 60 percent of the scientific effort is just somebody thinking, hmm, now if I make this machine, either a molecular machine or an electrophoresis gadget or we can use a restriction enzyme to cut this— So I think the technology and technology development is extremely important and, I would argue, even more important than hypothesis-driven research.

Hughes: That's an interesting statement from a person who has said that it's really been the conceptual side of science where you feel you've made the contribution, rather than the hands in the lab.

Riggs: I've done both. Maybe that's why I continue to be intrigued by just trying to think about new techniques, or if I see a new technique, thinking about how I can use it to develop a different technique. The goal that intrigues me is not only solving a problem we can't understand, but it's also developing a new technique, a new tool.

Hughes: And of course, the two are so interwoven, aren't they?

Riggs: That's right.

Hughes: I mean, you have a conceptual need, as you did in wanting to manipulate DNA efficiently, and that was really the origins of recombinant DNA.

Riggs: Yes, I think. I think I've said before, I forget exactly in what context, but I've called myself just a tool-maker. [laughter]

Hughes: Really?

Riggs: Yes. And it's kind of fun to think about that. They can either be conceptual tools, a new model--that's a tool--helps you think.

Hughes: Oh, okay, using the term very broadly.

Riggs: Or a new technique. That's a tool that allows you to think. Or a new piece of equipment. I did at one time try to develop equipment, and then recently have been trying to automate litigation-mediated PCR. So I don't mind thinking of myself as just a tool-maker.

Hughes: [laughs] In the broadest sense. [tape interruption]

Riggs: There is one aspect of epigenetics that's fun to talk about, so I would like to take a couple of minutes to talk about that.

[End Tape 7, Side A] ##
[Begin Tape 7, Side B]

Riggs: One of Mendel's laws of classical genetics depends on a gene functioning the same, whether it comes from the mother or father. That is not true for mammals. So one of Mendel's laws is just not strictly true for mammals. At least a hundred genes now function differently, depending on whether they come from the mother or the father.

Hughes: Significantly?

Riggs: Yes. For example, the gene for insulin-like growth factor, IGF-2, is not expressed at all if it comes from the mother. It's only expressed if it comes from the father.

Hughes: Why would that be?

Riggs: It's because in the gene that comes from the mother, the expression is suppressed by DNA methylation or this epigenetic mechanism, with 5-methyl cytosine.

There's a critical control element which is methylated or not. If the control region is methylated, it doesn't work, and then you get IGF expressed. So it's methylated if it comes from the father, and it's unmethylated if it comes from the mother. So the methylation state or the epigenetic information is different, depending on whether it comes from the mother or the father.

Hughes: What would be the purpose?

Riggs: Well, we don't know. So the answer is we don't know the purpose. Instead of answering that, let me point out one interesting fact. Some of the genes that are known to be affected by this epigenetic system, the DNA methylation system, function in the brain. So therefore, if your child misbehaves, it's theoretically possible to blame it on the father. [laughter] Or mother. Because we now know that, of those genes that are either on or off depending on whether they come from the mother or father, it's about 50-50. About half of them are expressed if they come from the mother, and about half expressed if they come from the father. So is the behavior of mammalian progeny affected by the methylation patterns in this system? The answer is, yes, they probably are. Now, I am speculating, and it's just sort of for the fun of it, but that's one of the puzzles that's been created by this understanding of epigenetics.

Hughes: Interesting. You said off tape that a large part of your activities nowadays involves administration. Would you like to say something about that, and then about the graduate program that you founded?

Riggs: Yes, I'd be happy to.

Starting in about 1982, I became chairman of the biology division. I remained chairman for most of the time, with a few years off around 1990. But I was chairman of biology for many years and gained a considerable amount of administrative experience. And then slowly over time, I've taken on more administrative roles. For example, I and some of my colleagues started a graduate school here at the City of Hope. I'm very happy with the way that turned out. So we now have a fully accredited graduate program with very good students. I think there are forty-five of them now.

Hughes: What fields are available for a doctorate?

Riggs: We do give a master's, but we primarily give a Ph.D. degree in biological sciences. The students that come here tend to be interested in applying research to medical problems. It's a wonderful setting for that, because you get the basic research—

Hughes: Yes, with the hospital right next door.

Riggs: —you get the basic research, right, in the context of the hospital. I think it works very well.

Hughes: Did you have any models in mind when you set up the program?

Riggs: We consulted with Scripps Institute. That was our primary model. So yes. But I think it was just Scripps.

I was actually the dean for, I don't know, four or five years. So both being chairman of biology and being the dean of the graduate school were very satisfying jobs. I enjoyed that. Then I also was associate research director for the City of Hope, and I did play a significant role in helping the City of Hope develop into a comprehensive NIH-supported cancer center. So we are now a comprehensive cancer center. I'm pleased I was able to help us grow in that direction. I think the total employees of City of Hope is about 2,500, so it's been steady growth, about 8 percent a year.

Hughes: Do you remember what it was when you first arrived?

Riggs: No, I don't really remember. But we did go back and plot the budget, and it's been a steady increase of 8 percent a year for the last twenty years.

Hughes: My heavens. That's quite a record.

Riggs: Yes. So I think things have gone very well here, so I'm reasonably pleased with, you might say, my career in administration.

Hughes: If you had to pick out one contribution, what would it be?

Riggs: That's a tough one. Can I pick two?

Hughes: All right. [laughter]

Riggs: [One] would be the somatostatin. I think somatostatin was a real milestone accomplishment, and I really enjoyed working with my colleagues also, the scientists and Bob Swanson; it was just great. But I would put right up there is my being the co-first to correctly identify a new genetic process, a new genetic mechanism. That's what I'm continuing to work on now. So that's held my interest all the time since I came to the City of Hope, and I'm still interested in that. I'm very pleased at the way that has sort of steadily increased from, say, two publications a year to now thousands of publications per year. I am just very pleased that cancer epigenetics is now considered to be the hottest new area, or one of the hottest new areas in cancer biology.

So I think those are two that I am very happy about.

Hughes: Well, I can well understand. Do you have any more comments to make?

Riggs: It's been a pleasure talking to you. [laughs]

Hughes: It's been a pleasure for me, too.

Riggs: So thank you.

Hughes: And I thank you.

[End Tape 7, Side B] ##
[End of Interview]

Appendix 1—Significant Research Accomplishments—Arthur D. Riggs, Ph.D.

1. Discovered bidirectional DNA replication in mammalian cells, and established many of the basic facts of mammalian DNA replication. (Publ. 2 on CV, 1968)
2. First to purify to homogeneity a gene regulatory protein, the lac repressor. (Publ. 3-4, 1968)
3. Developed the nitrocellulose membrane assay for DNA-protein interactions and established most of the basic parameters for the interaction of sequence-specific gene regulation proteins with DNA. (Publ. 6,7,8, 1970)
4. First to purify an activator protein (the catabolite gene activator protein) and established that an activator protein also can function by binding to DNA. (Publ. 11, 1971)
5. Identified and characterized a new enzyme, ribonuclease H. (Publ. 18, 1973)
6. First to covalently attach proteins to specific DNA sites by UV photocrosslinking. (Publ. 19, 1974)
7. First to recognize the importance to mammalian cells of nonspecific interactions of regulatory proteins with DNA. (Publ. 24, 1975, a citation classic)
8. First to propose that DNA methylation (5-methylcytosine) affects protein-DNA interactions and controls gene regulation during mammalian development. (publ. 20, 1975)
9. First to suggest that DNA methylation maintains X-chromosome inactivation (publ. 20, 1975).
10. First to propose the maintenance methylase concept as a new epigenetic mechanism for somatic inheritance. (Publ. 20, 47, 1975, 1980) A landmark paper in the field of epigenetics. In essence, I correctly identified a new kind of genetic code, now known to be essential for normal mammalian development.
11. First to establish that a chemically synthesized gene (the lac operator) can function in vivo. (Publ. 28, 1976)
12. First to use chemically synthesized "linkers" to aid recombinant DNA work. (Publ. 30, 1976) Now standard methodology.
13. First to obtain the expression in E. coli of functional mammalian peptides and proteins: the hormone somatostatin in 1977 and insulin in 1978. (Publ. 33, 38, 1977-78)
14. First to design and make a totally new gene with a sequence not found in nature, i.e., the first man-designed as well as man-made gene. (Publ. 33, 1977) (The chemically synthesized gene for somatostatin was not based on the natural DNA or mRNA sequence, which was unknown at the time.)
15. Co-first for establishment of the feasibility of oligonucleotide-directed mutation. (Publ. 35, 1978)
16. Independently developed the HpaII/MspI assay for DNA methylation. (Publ. 37, 1979)
17. First to deliberately change a protein using recombinant DNA and chemically synthesized DNA, i.e., first published work in the new field of protein engineering. (Publ. 50, 51, 52, 1981-82)
18. First successful production of monoclonal antibodies in bacteria. First deliberate alteration of antibodies by directed mutation techniques, i.e., first application of protein engineering to antibodies. (Publ. 58, 1984)
19. First deliberate domain shuffling and construction of a chimeric, plant-human enzyme. (Publ. 77, 1986)
20. New, simple method to prepare DNA for PCR. (Publ. 88. 1989) Now commonly used for forensic blood and tissue samples.
21. New model for cell memory, chromosome folding and enhancer function. (Publ. 91, 1990)
22. New, improved procedures for quantitative PCR and methylation analysis. (Publ. 92, 94-96, 98; 1989-90)

23. New method for genomic sequence, chromatin structure and methylation analysis: Ligation-mediated genomic sequencing. (Publ. 87, 1989)
24. New method for detecting specific single strand breaks at single-nucleotide resolution. (Publ. 102; 1991) A technical breakthrough for DNA adduct formation and mutation repair studies.
25. First determination of basic parameters (fidelity, etc.) for the epigenetic system dependent on maintenance methylase; partial explanation of X chromosome inactivation. (Publ. 100, 1990)
26. First X chromosome structure analysis by ligation-mediated PCR (Publ. 89, 97, 103; 1989-1991)
27. First *in vivo* DNase I protein footprint and chromatin fine-structure analysis of single-copy genes in mammalian cells (Publ. 101, 103; 1990-91).
28. Quantitative, allele specific assay for transcripts requiring just a few cells. (Publ. 105, 1991)
29. New technique for in vivo UV photofootprinting. (Publ. 108, 1992)
30. New experimental and theoretical approach to the study of cell memory and epigenetic mechanisms (Publ. 121; 1995)
31. Identification and evolutionary study of new interspersed repetitive elements in the human genome (Publ. 119,120, 124; 1995-96)
32. New method (TDPCR) for high-resolution analysis of chromatin and RNA (Publ. 137, 143; 1998-99)
33. First mouse mutation giving two stable, alternate phenotypes from one genotype in the same litter (Publ. 150, 2001)
34. New method for determining in vivo RNA-protein footprinting, RNA structure, and mRNA accessibility, which can guide ribozyme and siRNA site selection (publ 143, 145, 154; 2000-2001)
35 New theory suggesting that epigenetic silencing aids evolution by gene duplication (Publ. 163, 2003)

Appendix 2—Curriculum Vitae—Arthur D. Riggs, Ph.D.

Born: August 8, 1939, Modesto, California
A.B.: University of California at Riverside, 1961, Chemistry
Ph.D.: California Institute of Technology, Pasadena, 1966, Biochemistry

Professional Experience

1961-1966: Predoctoral Fellow, Biology Department, Calif. Inst. of Tech., Pasadena, CA
1966-1969: Postdoctoral Fellow, Salk Institute for Biological Studies, La Jolla, CA
1969-1974: Associate Research Scientist, Department of Molecular Biology, City of Hope National Medical Center, Duarte, CA
1974-1983: Senior Research Scientist, Dept. of Molecular Biology, City of Hope Med. Center
1978-Present: Adjunct Professor, University of Southern California, Los Angeles, CA
1979-1981: Associate Chairman, Division of Biology, City of Hope Medical Center
1981-1983: Chairman, Division of Biology, City of Hope Medical Center
1983-1987: Chairman, Division of Biology, Beckman Research Institute of the City of Hope
(In 1983 the City of Hope's research institute was renamed the Beckman Research Institute of the City of Hope.)
1994-2000: Chairman, Division of Biology, Beckman Research Institute of the City of Hope
1981-1987: Associate Director for Laboratory Research, City of Hope Cancer Center
1993-1995: Director, Shared Resources of City of Hope Cancer Center
1994-1998: Founding Dean, City of Hope Graduate School
1998-1999: Associate Director for Research, City of Hope Medical Center
1999-2000: Director of the Beckman Research Institute of the City of Hope
2000-Present: CEO and Director, Beckman Research Institute of the City of Hope

Honors & Awards

High honors in chemistry, University of California, Riverside, 1960
National Science Foundation, Predoctoral Fellow, 1961-1964
National Institutes of Health Postdoctoral Fellow, 1966-1969
Juvenile Diabetes Foundation Research Award, 1979
Gallery of Achievement Award, City of Hope, 1981
Invited applicant for NCI Outstanding Investigator Award, 1985
Distinguished Alumni Award, Univ. Calif., Riverside, 1988

Principal Research Interest:

Gene regulation and mammalian development, X-chromosome inactivation, DNA methylation, and epigenetic mechanisms.

PUBLICATIONS

1. Huberman, J.A. & Riggs, A.D. (1966) Autoradiography of chromosomal DNA fibers from Chinese hamster cells. Proc. Natl. Acad. Sci. USA 55:599-606.
2. Huberman, J. A. & Riggs, A.D. (1968) On the mechanisms of DNA replication in mammalian chromosomes. J. Mol. Biol. 32:327-341.
3. Riggs, A.D. & Bourgeois, S. (1968) On the assay, isolation, and characterization of the lac repressor. J. Mol. Biol. 34:36l-364.
4. Riggs, A.D., Bourgeois, S., Newby, R.F., & Cohen, M. (1968) DNA binding of the lac repressor. J. Mol. Biol. 34:365-368.
5. Riggs, A. D. (1969) On the lac repressor-operator interaction and the purification of the lac operator. Biophys. J. 9:84A.
6. Riggs, A.D., Suzuki, H., & Bourgeois, S. (1970) Lac repressor-operator interaction. I. Equilibrium studies. J. Mol. Biol. 48:67-83.
7. Riggs, A. D., Newby, R.F., & Bourgeois, S. (1970) Lac repressor-operator interaction. II. The effect of galactosides and other ligands. J. Mol. Biol.. 5l:303-3l4.
8. Riggs, A.D., Bourgeois, S., & Cohn, M. (1970) Lac repressor-operator interaction. III. Kinetic studies. J. Mol. Biol. 53:40l-417.
9. Bourgois, S. & Riggs, A.D. (1970) Lac repressor-operator interaction. IV. Assay and purification of operator DNA. Biochem. Biophys. Res. Commun. 38:348-354.
10. Lin, S. & Riggs, A.D. (1970) Lac repressor binding to DNA not containing the lac operator and to synthetic poly dAT. Nature 228:1184-1186.
11. Riggs, A. D., G. Reiness, & G. Zubay (1971) Purification and DNA binding properties of CAP, the catabolite gene activator protein. Proc. Natl. Acad. Sci. USA. 68:1222-1225.
12. Comings, D. & Riggs, A.D. (1971) Mechanisms of chromosome pairing, folding and function. Nature 233:48-50.
13. Lin, S. & Riggs, A.D. (1971) Lac repressor binding to operator analogues: comparison of poly[d(A-T)], poly[d(BrdU)], and poly[d(A-U)]. Biochem. Biophys. Res. Commun. 45:1542-1547.
14. Jobe, A., Riggs, A.D., & Bourgeois, S. (1972) Lac repressor-operator interaction. V. Characterization of super- and pseudo-wild-type repressors. J. Mol. Biol. 64:181-199.
15. Riggs, A. D., Lin, S., & Wells, R.D. (1972) Lac repressor binding to synthetic DNAs of defined nucleotide sequence. Proc. Natl. Acad. Sci. USA 69:761-764.
16. Lin, S. & Riggs, A.D. (1972) Lac repressor binding to non-operator DNA: detailed studies and a comparison of equilibrium and rate competition methods. J. Mol. Biol. 72:671-690.
17. Lin, S. & Riggs, A.D. (1972) Lac operator analogues: bromodeoxyuridine substitution in the lac operator affects the rate of dissociation of the lac repressor. Proc. Natl. Acad. Sci. USA 69:2574-2576.
18. Miller, H.I., Riggs, A.D., & Gill, G.N. (1973) Ribonuclease H (hybrid) in Eschericia coli: identification and characterization. J. Biol. Chem. 248:2621-2624.

19. Lin, S. & Riggs, A.D. (1974) Photochemical attachment of lac repressor to bromodeoxyuridine-substituted lac operator by ultraviolet radiation. Proc. Natl. Acad. Sci. USA 71:947-951.
20. Riggs, A.D. (1975) X inactivation, differentiation, and DNA methylation. Cytogenet. Cell Genet. 14:9-25.
21. Lin, S. & Riggs, A.D. (1975) A comparison of lac repressor binding to operator and to nonoperator DNA. Biochem. Biophys. Res. Commun. 62:704-7110.
22. Barkley, M.D., Riggs, A.D., Jobe, A., & Bourgeois, A. (1975) Interaction of effecting ligands with lac repressor and repressor-operator complex. Biochemistry 14:1700-1712.
23. Riggs, A.D. (1975) How genes are regulated. City of Hope Quarterly 4(4):3-7.
24. Lin, S. & Riggs, A.D. (1975) The general affinity of lac repressor for E.coli DNA: implications for gene regulation in procaryotes and eucaryotes. Cell 4:107-111.
25. Lin, S. & Riggs, A.D. (1976) The binding of lac repressor and the catabolite gene activator protein to halogen-substituted analogues of poly[d(A-T)]. Biochim Biophys. Acta 432:185-191.
26. Lin, S., Itakura, K., Rosenberg, J.M., Wilcos, G., Bahl, C., Wu, R., Narang, S., Dickerson, R., & Riggs, A.D. (1976) The interaction of chemically synthesized 2l base pair lac operator with the lac repressor. In, Molecular Mechanisms in the Control of Gene Expression, New York, Academic Press, pp. 143-158.
27. Lin, S., Lin, D., & Riggs, A.D. (1976) Histones bind more tightly to bromodeoxyuridine-substituted DNA than to normal DNA. Nucleic Acids Res. 3:2182-2191.
28. Heyneker, H.L., Shine, J., Goodman, H.M., Boyer, H.W., Rosenberg, J., Dickerson, R.E., Narang, S.A., Itakura, K., Lin, S., & Riggs, A.D. (1976) Synthetic lac operator DNA is functional in vivo. Nature 263:748-752.
29. Singer, J., Stellwagen, R.H., Roberts-Ems, J., & Riggs, A.D. (1977) 5-Methylcytosine content of rat hepatoma DNA substituted with bromodeoxyuridine. J. Biol. Chem. 252:5509-5513.
30. Scheller, R. H., Dickerson, R.E., Boyer, H.W., Riggs, A.D., & Itakura, K. (1977) Chemical synthesis of restriction enzyme recognition sites useful for cloning. Science 196:177-180.
31. Petersen, N.S., Riggs, A.D., & Seecof, R.L. (1977) A method for establishing cell lines from Drosophila melanogaster embryos. In. Vitro 13:36-40.
32. Rosenberg, J.M., Kallai, O.B., Kopka, M.L., Dickerson, R.E., & Riggs, A.D. (1977) Lac repressor purification without inactivation of DNA binding activity. Nucleic Acids Res. 4:567-571.
33. Itakura, K., Hirose, T., Crea, R., Riggs, A. D., Heyneker, H.L., Bolivar, F., Boyer, H.W. (1977) Expression in E. coli of a chemically synthesized gene for the hormone somatostatin. Science 198:1056-1062.
34. Koyama, H., Goodpasture, C., Miller, M.M., Teplitz, T.L., & Riggs, A.D. (1978) Establishment and characterization of a cell line from the American opossum (Didelphys virginiana). In Vitro 14:239-246.

35. Razin, A., Hirose, T., Itakura, K., & Riggs, A.D. (1978) Efficient correction of a mutation by use of chemically synthesized DNA. Proc. Natl. Acad. Sci. USA 75:4268-4270.
36. Riggs, A.D. & Itakura, K. (1978) Mammalian hormone production in bacteria. City of Hope Quarterly 7(2):3-6.
37. Singer, J., Roberts-Ems, J., & Riggs, A.D. (1979) Methylation of mouse liver DNA studied by means of the restriction enzymes MspI and HpaII. Science 203:1019-1021.
38. Goeddel, D.V., Kleid, D.G., Bolivar, F., Heyneker, H.L., Yansura, D.G., Crea, R., Hirose, Kraszewski, A., Itakura, K., & Riggs, A.D., (1979) Expression in E. coli of chemically synthesized genes for human insulin. Proc. Natl. Acad. Sci. USA 76:106-110.
39. Singer, J., Schnute, W.C., Shively, J.E., Todd, C.W., & Riggs, A.D. (1979) Sensitive detection of 5-methylcytosine and quantitation of the 5-methylcytosine/cytosine ratio in DNA by gas chromatography-mass spectrometry using multiple specific ion monitoring. Analyt. Biochem. 94:297-301.
40. Riggs, A.D. (1979) Synthetic DNA and medicine. Amer. J. Human Genet. 31:531-538.
41. Singer, J., Roberts-Ems, J., Luthardt, F.W., & Riggs, A.D. (1979) Methylation of DNA in mouse early embryos, teratocarcinoma cells and adult tissues of mouse and rabbit. Nucleic Acids Res. 7:2369-2385.
42. Riggs, A.D., Itakura, K., Crea, R., Hirose, T., Kraszewski, A., Goeddel, D., Kleid, D., Yansura, D., Bolivar, F., & Heyneker, H. (1979) Synthesis, cloning, and expression of hormone genes in eschericia coli. Rec. Prog. Hormone Res. 30:26l-276.
43. Riggs, A.D., Itakura, K., Hirose, T., Kraszewski, A., Crea, R., Goeddel, D., Kleid, D., Yansura, D., Bolivar, R., & Heyneker, H. (1979) Chemical DNA synthesis as an approach to peptide synthesis: the human insulin project. In, "Peptides: Structure and Biological Function." Ed. by E. Gross and J. Meienhofer. Pierce Chemical Co.
44. Kallai, O. B., Rosenberg, J.M., Kopka, M.L., Takano, T., Dickerson, R.E., Kan, J., & Riggs, A.D. (1980) Large-scale purification of two forms of lac operator. Biochim. Biophys. Acta 606:113-124.
45. O'Gorman, R.B., Rosenberg, J.M., Kallai, O., Dickerson, R.E., Itakura, K., Riggs, A.D., & Matthews, K.S. (1980) Equilibrium binding of inducer to lac repressor-operator DNA complex. J. Biol. Chem. 255:10107-10114.
46. Itakura, K. & Riggs, A.D. (1980) Chemical DNA synthesis and recombinant DNA studies. Science 209:1401-1405.
47. Razin, A. & Riggs, A.D. (1980) DNA methylation and gene function. Science 210:604-610.
48. Riggs, A.D. (1981) Bacterial production of human insulin. (Levine Festschrift, 1980) Diabetes Care 4:64-68.
49. Riggs, A.D. & Reilly, J.G. (1981) 5-Methylcytosine, gene regulation, and cancer. City of Hope Quarterly 11(1): 3-5.
50. Wetzel, R., Kleid, D.G., Crea, R., Heyneker, H.L., Yansura, D.G., Hirose, T., Kraszewski, A., Riggs, A.D., Itakura, K., & Goeddel, D.V. (1981) Expression in E. coli of a chemically synthesized gene for a "mini-C" analog of human proinsulin. Gene 16:63-71.

51. Dalbadie-McFarland, G., Cohen, L.W., Riggs, A.D., Morin, C., Itakura, K., & Richards, J.H. (1982) Oligonucleotide-directed mutagenesis as a general and powerful method for studies of protein function. Proc. Natl. Acad. Sci. USA 79:6409-6413.
52. Cohen, L.W., Molin, C., Itakura, K., Riggs, A.D., Dalbadiie-McFarland, G., & Richards, J.H. (1982) Proteins to order: use of synthetic DNA to generate site-specific mutations. In, Proceedings of 28th macromolecular symposium, IUPAC, Amherst, Massachusetts. P. 335.
53. Singer-Sam, J., Simmer, R.L., Keith, D.H., Shively, L., Teplitz, M., Itakura, K., Gartler, S.M., & Riggs, A.D. (1983) Isolation of a cDNA clone for human X-linked 3-phosphoglycerate kinase by use of a mixture of synthetic oligodeoxyribonucleotides as a detection probe. Proc. Natl. Acad. Sci. USA 80: 802-806.
54. Riggs, A.D. & Jones, P.A. (1983) Methylcytosine, gene regulation, and cancer. Adv. Cancer Res. 40: 1-30.
55. Gartler, S.M. & Riggs, A.D. (1983) Mammalian X-chromosome inactivation. Annu. Rev. Genet. 17:153-190.
56. Shively, J.E., Simmer, R., Pande, H., Yang, J., Wagener, C., Riggs, A.D., & Todd, C.W. (1984) Structural studies on carcinoembryonic antigen and molecular cloning of carcinoembryonic antigen using mixed synthetic oligonucleotide probes. Progress in Cancer Research and Therapy 29: 147-157.
57. Riggs, A.D., Singer-Sam, J., Keith, D., & Carr, B.I. (1984) DNA methylation, X inactivation, and cancer. In, Advances in Gene Technology: Human Genetic Disorders. Proceedings of the Sixteenth Miami Winter Symposia (ICSU Short Reports, Vol. 1, pp. 32-35).
58. Cabilly, S., Riggs, A.D., Pande, H., Shively, J.E., Holmes, W., Wetzel, R., & Heyneker, H. (1984) Generation of antibody activity from immunoglobulin peptide chains produced by E.coli. Proc. Natl. Acad. Sci. USA 81:3273-3277.
59. Pande, H., Baak, S.W., Riggs, A.D., Clark, B.R., Shively, J.E., and Zaia, J.A. (1984) Cloning and physical mapping of a gene fragment coding for a 64K-dalton major late antigen of human cytomegalovirus. Proc. Natl. Acad. Sci. USA 81: 4965-4949.
60. Riggs, A.D., Itakura, K., & Boyer, H.W. (1984) From somatostatin to human insulin. In, Recombinant DNA Products: Interferon-Insulin Growth Hormone. Ed., A. P. Bollon. (Boca Raton, Florida, CRC Press, Inc.) pp. 37-45.
61. Carr, B.I., Reilly, J.G., Smith, S.S., Winberg, C., & Riggs, A.D. (1984) The tumorigenicity of 5-azacytidine in the male Fischer rat. Carcinogenesis 5:1583-1590.
62. Razin, A., Cedar, H., & Riggs, A.D. (1984) DNA Methylation: Biochemistry and Biological Significance. Eds, Razin, A., Cedar, H., & Riggs, A.D., New York, Springer-Verlag. pp. 1-10.
63. Riggs, A.D. (1984) X inactivation, DNA methylation, and differentiation revisited. In, DNA METHYLATION, Biochemistry and Biological Significance. Eds., Razin, A., Cedar, H., & Riggs, A.D., New York, Springer-Verlag. pp. 269-278.

64. McCarrey, J.R., Pande, H., Churchill, M.A., Welsing, M.W., Zaia, J.A., & Riggs, A.D. (1984) Identification by in situ cytohybridization of human cells infected with HCMV. In, "Herpesviruses". Ed., F. Rapp. Alan R. Liss, Inc. pp. 477-486.
65. Dalbadie-McFarland, G., Neitzel, J., Riggs, A.D., & Richards, J.H. (1984) Studies of protein function by various mutagenic strategies: beta-lactamase. In, Enzyme Engineering, 7. (Annals of New York Academy of Scientists, Vol. 434) pp. 232-238.
66. Singer-Sam, J., Keith, D.H., Tani, K., Simmer, R.L., Shively, L., & Lindsay, S., & Riggs, A.D. (1984) Sequence of the promoter region of the gene for human X-linked 3-phosphoglycerate kinase. Gene 32:409-417.
67. Keith, D.H., Teplitz, R.L., & Riggs, A.D. (1984) Metaphase synchronization and chromosome preparation from the OK opossum cell line having a potentially isolatable X chromosome. In Vitro 20:833-836.
68. Dalbadie-McFarland, G., Riggs, A.D., & Richards, J.H. (1984) Directed mutagenesis as a technique to study protein function: application to beta-lactamase. Biochem. Soc. Trans. (England) 12:226-228.
69. Carr, B.I., Reilly, J.G., & Riggs, A.D. (1984) 5-Azacytidine. Promoting activity for rat hepatocellular carcinoma. IARC Sci Pub. (France) 56:409-412.
70. Williamson, R.L. & Riggs, A.D. (1985) 5-Aza-2'-deoxycytidine does not cause recessive lethal mutations in drosophila melanogaster. Drosophila Inform. Serv. 61:184-185.
71. Lindsay, S., Monk, M., Holliday, R., Huschtscha, L., Davies, K.E., Riggs, A.D., & Flavell, R.A. (1985) Differences in methylation on the active and inactive human X chromosomes. Ann. Hum. Genet. 49:115-127.
72. Riggs, A.D., Singer-Sam, J., & Keith, D.H. (1985) Methylation of the PGK promoter region and an enhancer way-station model for X-chromosome inactivation. In, Biochemistry and Biology of DNA Methylation, Alan Liss, N.Y., pp. 211-222. (Progress in Clinicical & Biological Research, Vol. 198, Ed. by G.L. Cantoni & A. Razin.)
73. Cabilly, S. & Riggs, A.D. (1985) Immunoglobulin transcripts and molecular history of a hybridoma that produces antibody to carcinoembryonic antigen. Gene 40:157-161.
74. Zucker, K.E., Riggs, A.D., & Smith, S.S. (1985) Purification of human DNA (cytosine-5-)-methyltransferase. J. Cell Biochem. 29:337-349.
75. McCarrey, J. & Riggs, A.D. Determinator-inhibitor pairs as a mechanism for threshhold setting in development: a possible function for pseudogenes. Proc. Natl. Acad. Sci. USA (1986) 83:679-683.
76. Mori, N., Singer-Sam, J., & Riggs, A.D. (1986) Evolutionary conservation of the substrate binding cleft of phosphoglycerate kinases. FEBS Lett. 204:313-317.
77. Mas, M.T., Chen, C.Y., Hitzeman, R.A., & Riggs, A.D. (1986) Active human-yeast chimeric phosphoglycerate kinases engineered by domain interchange. Science 233:788-790.
78. Keith, D.H., Singer-Sam, J., & Riggs, A.D. (1986) Active X-chromosome DNA is unmethylated at eight CCGG sites clustered in a GC-rich island at the 5' end of the gene for phosphoglycerate kinase. Mol. Cell Biol. 6:4122-4125.

79. Mori, N., Singer-Sam, J., Lee, C-Y., & Riggs, A.D. (1986) The nucleotide sequence of a cDNA clone containing the entire coding region for mouse X-chromosome-linked phosphoglycerate kinase. Gene 45:275-280.
80. Thompson, J.A., Pande, H., Paxton, R.J., Shively, L., Arunachalam, P., Simmer, R.L., Todd, C.W., Riggs, A.D., & Shively, J.E. (1987) Molecular cloning of a gene belonging to the carcinoembryonic antigen (CEA) gene family. Proc. Natl. Acad. Sci. 84:2965-2969.
81 Vogelstein, B., Fearon, E.R., Hamilton, S.R., Preisinger, A.C., Willard, H.F., Michelson, A.M., Riggs, A.D., & Orkin, S.H. (1987) Clonal analysis using recombinant DNA probes from the X chromosome. Cancer Res. 47:4806-4813, 1987.
82. Mas, M.T., Resplandor, Z.E., & Riggs, A.D. (1987) Site-directed mutagenesis of glutamate-190 in the hinge region of yeast 3-phosphoglycerate kinase: implications for the mechanism of domain movement. Biochemistry 26:5369-5377, 1987
83. Yang, T.P., Singer-Sam, J., Flores, J., & Riggs, A.D. (1987) Multiple factors bind to multiple DNA sites in the 5' region of human X-linked PGK. Somat. Cell Mol. Biol. 14:461-472.
84. Carr, B.I., Rabbar, S., Asmeron, Y., Riggs, A.D., & Winberg, C. (1988) Carcinogenicity and haemoglobin synthesis induction by cytidine analogs. Brit. J. Cancer 57:395-402.
85. Neumaier, M., Zimmermann, W., Shively, L., Hinoda, Y., Riggs, A. D., & Shively, J. E. (1988) Characterization of a cDNA clone for the nonspecific cross-reacting antigen (NCA) and a comparison of NCA and carcinoembryonic antigen. J. Biol. Chem. 263: 3202-3207
86. Riggs, A.D. (1989) DNA methylation and cell memory. Cell Biophysics 15: 1-13
87. Pfeifer, G., Steigerwald, S., Mueller, P., Wold, B., and Riggs, A. D. (1989) Genomic sequencing and methylation analysis by ligation-mediated PCR. Science 246: 810-813.
88. Singer-Sam, J., Tanguay, R.L., and Riggs, A.D. (1989) Use of Chelex to Improve the PCR signal from a small number of cells. Amplifications 1: 11.
89. Shively, J.E., Hinoda, Y.,Hefta, L.J.F., Neumaier, M., Hefta, S.A., Shively, L., Paxton, R.J., and Riggs, A.D. (1989) Molecular cloning of members of the carcinoembryonic antigen gene family. in The Carcinoembryonic Antigen Gene Family. eds. A. Yachi and J.E. Shively. Elsevier Science Publishers, pp. 97-110.
90. Riggs, A.D. (1990) Marsupials and Mechanisms of X Chromosome Inactivation. AUSTRALIAN J. ZOOL. (Boden Conference, special issue). 37:419-441
91. Riggs, A. D. (1990) DNA methylation and late replication probably aid cell memory, and type I DNA reeling could aid chromosome folding and enhancer function. Phil. Trans. R. Soc. Lond. 326: 285-297.
92. Singer-Sam, J., Yang, T.P., Mori, N., Tanguay, R.L., Le Bon, J.M., Flores, J.C., and Riggs, A.D. (1990) DNA methylation in the 5' region of the mouse PGK-1 gene and a quantitative PCR assay for methylation. In UCLA Symposia on Molecular and Cellular Biology, New Series, Vol. 128. "Nucleic Acid Methylation". Eds. G. Clawson, D. Willis, A. Weisbach, and P. Jones. Alan Liss, New Nork., pp. 285-298
94. Singer-Sam, J., Robinson, M., Belve', A.R., Simon, M., and Riggs, A.D. (1990) Measurement by quantitative PCR of Changes in HPRT, PGK-1, PGK-2, APRT, MTase, and ZFY gene transcripts during mouse spermatogenesis. Nucleic Acids Res. 18: 1255-1259.93.

Neumaier, M., Shively, L., Chen, F., Gaida, F., Ilgen, C., Paxton, R.J., Shively, J.E., and Riggs, A.D. (1990) Cloning of the genes for T84.66, an antibody which has a high specificity and affinity for carcinoembryonic antigen (CEA) and expression of chimeric human/mouse T84.66 genes in myeloma and CHO cells. Cancer Research 50:2128-2134.

95. Singer-Sam, J., Tanguay, R.L., Le Bon, J.M., and Riggs, A.D. (1990) A HpaII-PCR method suitable for quantitative methylation analysis of DNA from a small number of cells. Nucleic Acids Res 3: 687.
96. Steigerwald, S.D., Pfeifer, G.P., and Riggs, A.D. (1990) Ligation-mediated PCR improves the sensitivity of methylation analysis by restriction enzymes and detection of specific DNA strand breaks. Nucleic Acids Res.18:1435-1439.
97. Pfeifer, G.P., Tanguay, R.L., Steigerwald, S.D., and Riggs, A.D. (1990) In vivo footprint and methylation analysis by genomic sequencing: comparison of active and inactive X chromosomal DNA at the CpG island and promoter of human PGK-1. Genes and Development 4:1277-1287.
98. Singer-Sam, J., Grant, M., Le Bon, J.M., Okuyama, K., Chapman, V., Monk, M., and Riggs, A.D. (1990) Use of a HpaII-PCR assay to study DNA methylation in the Pgk-1 CpG island of mouse embryos at the time of X inactivation. Mol. Cell Biol. 10:4987-4989.
99. Watson, J. M., Spencer, J. A., Riggs, A. D., and Graves, J. A. M. (1990) The X chromosome of monotremes shares a highly conserved region with the eutherian and marsupial X chromosomes despite the absence of X chromosome inactivation. Proc. Natl. Acad. Sci. USA. 87:7125-7129.
100. Pfeifer, G.P., Steigerwald, S.D., Hansen, R.S., Gartler, S.M., and Riggs, A.D. (1990) PCR-aided genomic sequencing of an X-linked CpG island: Methylation patterns suggest clonal inheritance, CpG site autonomy, and an explanation of activity state stability. Proc. Natl. Acad. Sci. USA. 87:8252-8256.
101. Tanguay, R.L., Pfeifer, G.P., and Riggs, A.D. (1990) PCR-aided DNase1 footprinting of single copy gene sequences in permeabilized cells. Nucl. Acids Res. 18:5902
102. Pfeifer, G.P., Drouin, R., Riggs, A.D., and Holmquist, G.P. (1991) Detection of pyrimidine (6-4) pyrimidone photoproducts in a human single copy gene by PCR-aided genomic sequencing. Proc. Natl. Acad. Sci. USA, 88:1374-1378.
103. Pfeifer, G.P., and Riggs, A.D. (1991) Chromatin differences between active and inactive X chromosomes revealed by genomic footprinting of permeabilized cells using DNase I and ligation-mediated PCR. Genes and Develop. 5:1102-1113.
104. Watson, J.M., Spencer, J.A., Riggs, A.D., and Graves, J.A.M. (1991) Sex chromosome evolution: Platypus gene mapping suggests that part of the human X was originally autosomal. Proc. Natl. Acad. Sci., 88:11256-11260.
105. Singer-Sam, J., LeBon, J.M., Dai, A., and Riggs, A.D. (1992) A sensitive, quantitative assay for measurement of allele-specific transcripts differing by a single nucleotide. PCR Methods Appl., 1:160-163.

106. Singer-Sam, J., Goldstein, L., Dai, A., Gartler, S.M., and Riggs, A.D. (1992) A potentially critical Hpa II site of the X-linked PGK-1 gene is unmethylated prior to the onset of meiosis of human oogenic cells. Proc. Natl. Acad. Sci. USA, 89: 1413-1417
107. Riggs, A.D., and Pfeifer, G.P. (1992) X chromosome inactivation and cell memory. Trends Genet., 8: 169-174
108. Pfeifer, G.P., Drouin, R., Riggs, A.D., and Holmquist, G.P. (1992) Binding of transcription factors creates hot spots for UV photoproducts in vivo. Mol. Cell Biol., 12: 1798-1804
109. Watson, J.M., Riggs, A.D., and Graves, J.A. (1992) Gene mapping studies confirm homology between the platypus X and echidna X1 chromosomes and identify a conserved ancestral monotreme X chromosome. Chromosoma 101: 596-601
110. Singer-Sam, J., Chapman, V., LeBon, J.M., and Riggs, A.D. (1992) Parental imprinting studied by allele-specific primer extension after PCR: paternal X-linked genes are transcribed prior to preferential paternal X chromosome inactivation. Proc. Natl. Acad. Sci. USA. 89: 10469-10473.
111. Tormanen, V.T., Swiderski, P.M., Kaplan, B.E., Pfeifer, G.P., and Riggs, A.D. (1992) Extension product capture improves genomic sequencing and DNaseI footprinting by ligation-mediated PCR. Nucleic Acids Res. 20: 5487-5488
112. Itakura K; Tadaaki H; Crea R; Riggs AD; Heyneker HL; Bolivar F; Boyer HW (1992) Expression in Escherichia coli of a chemically synthesized gene for the hormone somatostatin. 1977 [classical article]. Biotechnology (United States) 24: 84-91
113. Pfeifer, G.P., and Riggs, A.D. (1993) Genomic Footprinting by Ligation-Mediated PCR. Methods Mol. Biol. 16: 153-168.
114. Pfeifer, G.P., and Riggs, A.D. (1993) Genomic Sequencing. Methods Mol Biol, 23: 169-181
115. Singer-Sam, J., and Riggs, A.D. (1993) X chromosome inactivation and DNA methylation. in DNA methylation: Molecular biology and biological significance. eds. J. Jost and H. Saluz, Birkhauser Verlag, Berlin.
116. Pfeifer, G.P., Singer-Sam, J., and Riggs, A.D. (1993) PCR assays for methylation and chromosome structure. Methods Enzymol. 225: 567-583
117. Singer-Sam, J., and Riggs, A.D. (1993) Application of PCR to the quantitative measurement of mRNA levels: the quantitative RT-PCR SNuPE assay. Methods Enzymol. 225: 344-351
118. Tommasi, S., LeBon, J.M., Riggs, A.D. and Singer-Sam, J. (1993) Methylation Analysis By Genomic Sequencing Of The 5' Region of the Mouse Pgk-1 Gene and a Cautionary Note Concerning the Method. Somat. Cell Mol. Genet. 19:529-541.
119. Smit, A.F.A., and Riggs, A.D. (1995) MIRs are classic, tRNA-derived SINEs that amplified before the mammalian radiation. Nucleic Acids Res. 23:98-102
120. Smit, A.F.A., Toth, G., Riggs, A.D.,and Jurka, J. (1995) Identification of the ancestral LINE1 repetitive sequences in the human genome. J. Mol. Biol. 246:401-417
121. Hershkovitz, M., and Riggs, A.D. (1995) Metaphase chromosome analysis by ligation mediated PCR: heritable chromatin structure and differences between active and inactive X chromosomes. Proc. Natl. Acad. Sci. USA 92: 2379-2383.

122. LeBon, J.M., Tam, P.P.L., Singer-Sam, J., Riggs, A.D., and Tan, S.S. (1995) Mouse endogenous X-linked genes do not show lineage-specific delayed inactivation during development. Genetical Res. 65:223-227.
123. Mayes-Hoopes, L.L., Bolen, J.B., Riggs, A.D., and Singer-Sam, J. (1995) Preparation of spermatogonia, spermatocytes, and round spermatids bor analysis of gene expression using fluorescence activated cell sorting. Biology of Reproduction, 53:985-993.
124. Smit, A.F., and Riggs, A.D. (1996) Tiggers and other DNA transposon fossils in the human genome. Proc. Natl. Acad. USA 93:1443-1448.
125. Shemer, R., Birger, Y., Kafri, T., Dean, W.L., Reik, W., Riggs, A.D., and Razin, A., and (1996) Dynamic methylation adjustment and counting as part of imprinting mechanisms. Proc. Natl. Acad. Sci. USA. 93: 6659-6664.
126. Pfeifer, G.P. and Riggs, A.D. (1996) Genomic sequencing by ligation-mediated PCR. Molecular Biotechnology. 5: 281-288
127. Riggs, A.D. and Porter, T.N. (1996) Overview of Epigenetic Mechanisms. In Epigenetics Mechanisms of Gene Regulation. eds. Russo, E., Martienssen, R. and Riggs, A.D. Cold Spring Harbor Press, pp29-45.
128. Riggs, A.D. and Porter, T.N. (1996) X chromosome inactivation and epigenetic mechanisms. In Epigenetics Mechanisms of Gene Regulation. eds. Russo, E., Martienssen, R. and Riggs, A.D. Cold Spring Harbor Press, pp231-248
129. Russo, E., Martienssen, R. and Riggs, A.D. ed. (1996) Epigenetics Mechanisms of Gene Regulation. Cold Spring Harbor Press.
130. Hershkovitz, M. and Riggs, A.D. (1997). Ligation-mediated PCR for chromatin-structure analysis of interphase and metaphase chromosomes. Methods: A Companion to Methods in Enzymology, 11:253-263
131. Riggs, A.D. and Pfeifer G.P. (1997) PCR-aided genomic footprinting. In "In Vivo Footprinting," Series Title: Advances in Mol. and Cell Biol. ed I.L. Cartwright. JAI Press, Greenwich, Conn., Vol. 21, pp47-72
132. Komura, J., LeBon, J.M., Riggs, A.D., and Singer-Sam, J. (1997) In vivo UV and DMS footprinting of expressed and silent forms of the mouse Xist gene promoter. J. Biol. Chem. 272:10975-10980
133. Shemer, R., Birger Y., Riggs, A.D, and Razin, A. (1997). Structure of the imprinted mouse Snrpn gene and establishment of its parental-specific methylation pattern. Proc. Natl. Acad. Sci. USA 94:10267-10272
134. Riggs, A.D., and Hershkovitz, M. (1997) X chromosome inactivation and heritable chromatin structure. In Nuclear organization, chromatin structure, and gene expression. eds. R. van Driel and A.P. Otte, Oxford University Press, New York, pp197-222
135. Riggs, A.D., Xiong, Z., Wang, L., Lebon, J.M. (1997) Methylation dynamics, epigenetic fidelity, and X chromosome structure. Novartis Foundation Symp. 214:214-225
136. Xiong, Z., Tsark, W., Singer-Sam J., and Riggs A.D. (1998) Differential replication timing of X-linked genes measured by a novel method using single-nucleotide primer extension. Nucleic Acids Res. 26: 684-686

137. Komura, J., and Riggs, A.D. (1998) Terminal deoxynucleotidyl transferase-dependent PCR, a new, more sensitive approach to genomic footprinting and adduct detection. Nucleic Acids Res. 26:1807-1811.
138. Riggs, A.D., Singer-Sam, J., and Pfeifer, G.P. (1998). In vivo footprint and chromatin analysis by LMPCR. In Chromatin: A Practical Approach. ed H. Gould. Oxford Univ. Press pp 79-109
139. Denissenko, M.F., Koudriakova, T.B. Smith, L., O'Connor, T.R., Riggs, A.D., and Pfeifer, G.P. (1998) The p53 codon 249 mutational hotspot in hepatocellular carcinoma is not related to selective formation or persistence of aflatoxin B_1 adducts. Oncogene 17, 3007-3014
140. Pfeifer, G.P., Chen, H.H., Komura, J., and Riggs, A.D. (1999) Chromatin structure analysis by ligation-mediated PCR and terminal transferase-mediated PCR. Methods in Enzymol., 304: 548-571
141. Chen, H.H., Castanotto, D., Rossi, J.J., and Riggs. (1999) RNA analysis by terminal transferase-dependent PCR. in "Intracellular Ribozyme Applications: Principles and Protocols," J.J. Rossi and L. Couture, Eds., Horizon Scientific Press, Norfolk, UK
142. Scherr, M., LeBon, J.M., Riggs, A.D., and Rossi, J.J. (1999) The use of cell extracts and antisense deoxyribo-oligonucleotides for identifying ribozyme cleavage sites on messenger RNAs in "Intracellular Ribozyme Applications: Principles and Protocols," J.J. Rossi and L. Couture, Eds., Horizon Scientific Press, Norfolk, UK
143. Chen, H.H., Castanotto, D., LeBon, J., Rossi, J.J. and Riggs, A.D. (2000) *In vivo*, high-resolution analysis of yeast and mammalian RNA-protein interactions, RNA structure, RNA splicing, and Ribozyme cleavage by use of terminal transferase-mediated PCR. Nucl. Acids Res. 28: 1656-1664
144. Buettner, V.L., LeBon, J.M., Gao, C., Riggs, A.D. and Singer-Sam, J. (2000) Use of terminal transferase-dependent antisense RNA amplification to determine the transcription start site of the Snrpn gene in individual neurons. Nucl. Acids Res. 28: e25
145. Scherr, M, Reed, M., Huang, C.F., Riggs, A.D, and Rossi, J.J. (2000) Oligonucleotide-scanning in extracts predicts *in vivo* ribozyme efficiency for reduction of murine DNA-methyltransferase. Molecular Therapy 2:26-38
146. Dai, S.M., Chen, H.H., Chang, C., Riggs, A.D., and Flanagan, S.D. (2000) Ligation-mediated PCR using an automated, hot-start procedure: In vivo footprinting and genomic sequencing using nonradioactive detection. Nature Biotech. 18:1108-1111
147. Kontaraki, J., Chen, H.H., Riggs, A.D, and Bonifer, C. (2000) Chromatin fine structure profiles for a developmentally regulated gene: reorganization of the lysozyme locus prior to trans-activator binding and gene expression. Genes Develop, 14:2106–2122.
148. Rusmintratip, V., Riggs, A.D., and Sowers, L.C. (2000) Examination of the DNA Substrate Selectivity of DNA Cytosine Methyltransferases Using Mass Tagging. Nucl. Acids Res. 28:3594-3599.
149. O'Connor, T., Chen, H.H., Dai, S.M., Flanagan, S.D., Akman, S.A., Holmquist, G.P., Rodriguez, H., and Riggs, A.D. (2001) "Mapping DNA Damage at Nucleotide Resolution in

Mammalian Cells" In Oxidative Stress and Aging: Advances in Basic Science, Diagnostics, and Intervention, World Scientific Publishing, Inc. In Press
150. Reed M.R., Huang, C.F., Riggs, A.D. and Mann, J.R. (2001) A complex duplication created by gene targeting at the imprinted H19 locus causes two distinct classes of methylation and correlated Igf2 expression phenotypes. Genomics, 74:186-196
151. Komura, J., Ikehata, H., Hosoi, Y., Riggs, A.D., and Ono, T. (2001) Psoralen Cross-Links at the Nucleotide Level in Mammalian Cells: Suppression of Cross-Linking at Transcription Factor- or Nucleosome-Binding. Biochemistry 40:4096-4105
152. Chen, H.H., Kontaraki, J., Bonifer, C., and Riggs, A.D. (2001) Terminal transferase-dependent PCR (TDPCR) for in vivo footprinting of vertebrate cells. Science STKE 77:PL1
153. Reed, M.R., Riggs, A.D., and Mann, J.R. (2001) Deletion of a direct repeat element has no effect on Igf2 and H19 imprinting. Genomics 74: 873-876
154. Scherr, M., LeBon, J., Castanotto, D., Cunliffe, H.E., Meltzer, P.S., Ganser, A., Riggs, A.D, and Rossi, J.J. (2001) Detection of antisense and ribozyme accessible sites on native mRNAs: application to NCOA3 mRNA. Mol. Therapy 4:454-460.
155. Chong, S., Riggs, A.D., and Bonifer, C. (2002) The chicken lysozyme chromatin domain contains a second, widely-expressed gene. Nucl. Acids Res., 30: 463-467
156. Chong S., Kontaraki J., Bonifer C. and Riggs A.D. (2002) A functional chromatin domain does not resist X chromosome inactivation: Silencing of cLys correlates with methylation of a dual promoter-replication origin. Mol. Cell. Biol. 22:4667-4676
157. Tagoh H., Himes R., Clarke, D., Leenen P., Riggs A.D., Hume D. and Bonifer C. (2002) Transcription factor complex formation and chromatin fine structure alterations at the murine c-fms (CSF-1 receptor) locus during the differentiation of purified early myeloid precursor cells into activated macrophages. Genes Develop. 16:1721-1737
158. Bane, T.K., Leblanc, J.F., Lee, T.D., and Riggs, A.D. (2002) DNA affinity capture and protein profiling by SELDI-TOF mass spectrometry: Effect of DNA methylation. Nucl. Acids Res. 30:1-6
159. Dai, S.M., Chang, C., O'Connor, T.R., Holmquist, G.P., Riggs, A.D., and Flanagan, S. (2002) Automated Ligation-Mediated PCR with Fluorescent Detection: DNA Damage and Repair in Multiple P53 Exons. Biotechniques 33:1090-1097
160. Riggs, A.D. (2002) X chromosome inactivation, differentiation and DNA methylation revisited, with tribute to Susumu Ohno, Cytogenet. Genome Res., Vol. 99, 17-24
161. Shively, L., Chang, L., LeBon, J.M., Liu, Q., Riggs, A.D. and Singer-Sam, J. (2003) A real-time PCR assay for quantitative mismatch detection. Biotechniques 34:498-504
162. Lefevre, P., Melnik, S., Wilson, N., Riggs, A.D. and Bonifer, C (2003) Developmentally regulated recruitment of transcription factors and chromatin modification activities to chicken lysozyme cis-regulatory elements in vivo. Mol. Cell. Biol. 23:4386-4400
163. Rodin, S.N. and Riggs, A.D. (2003) Epigenetic silencing may aid evolution by gene duplication. J. Mol. Evol., 56:718-729

164. Allen, E., Spiten, E., Tong, F., Kraft, P., Horvath, S., Riggs, A.D., and Marahrens, Y. (2003) Chromosomal context identifies two categories of allelically excluded genes. Proc. Natl. Acad. Sci USA, 100: 9940-9945

165. Melnik, S., Tagoh, H., Lefevre, P., Chong, S., Riggs, A.D., and Bonifer, C. (2004) Dynamic reorganization of chromatin structure and selective DNA-demethylation prior to stable enhancer complex formation during differentiation of primary hematopoietic cells in vitro. Blood 103:2950-2955

166. Riggs, A.D., and Xiong, Z. (2004) Methylation and epigenetic fidelity. Proc. Nat. Acad. Sci. USA, 101: 4-5

167. Chen HH, Castanotto D, LeBon J, Rossi JJ, Riggs AD. (2004) In vivo detection of ribozyme cleavage products and RNA structure by use of terminal transferase-dependent PCR. Methods Mol Biol. 252:109-124.

168. Kim, D.H., Lee, K.B., Riggs, A.D., and Rossi, J.J. (2004) La is an essential component of RNAi in mammalian cells. EMBO in revision.

169. Chen, Z., Mann, J., Hsieh, C.L., Riggs, A.D. and Chedin, F (2004) Physical and functional interactions between the human DNMT3L protein and members of the *de novo* methyltransferase family. Submitted

Manuscripts in preparation:

Chang, C., LeBon, J.M., Komura, J., Singer-Sam, J., and Riggs, A.D. High resolution in vivo chromatin accessibility assay by allele-specific LMPCR.

Wang, L., LeBon, J.M., and Riggs, A.D. Differential DNase I accessibility between the active and inactive X chromosome: implications for chromatin structure.

Chen, H.H., and Riggs, A.D. TDPCR analysis of Xist RNA

Huang, J., and Riggs, A.D. Factors affecting epigenetic silencing and reactivation of various GFP containing transgenes.

Reed, M.R., Chen, Z., Riggs, A.D. and Mann, J.R. DNA methylation and DNA methyltransferase levels in primordial germ cells.

Reed, M.R., Riggs A.D. and Mann, J.R. Erasure and establishment of genomic imprinting in primordial germ cells

Proteins binding to methylated and unmethylated Xist promoter

Proteins binding specifically to Xist RNA

A novel tissue-specific splicing variant for Dnmt 3B

Kim, D.H., Langlois, M.A., Lee, K.B., Puymirat, J., Riggs, A.D. and Rossi J.J., (2004) An alternative splicing defect in Myotonic Dystrophy type 1 (DM1) myoblasts can be rescued by overexpression of hnRNP H. in preparation.

Work supported by ADR grants but name not added to paper.

Smit, A.F.A. (1993) Identification of a new, abundant superfamily of mammalian LTR-transposons, Nucleic Acids Res. 21: 1863-1872

Cooney, C. A. (1993) Are somatic cells inherently deficient in methylation metabolism? A proposed mechanism for DNA methylation loss, senescence, and aging. Growth, Development and Aging, 57: 261-273

Cooney, C.A. (1994) Techniques and High Resolution DNA Size Markers for Pulsed Field Electrophoresis. Molecular Biotechnology 2:119-127.

Buzin, C.H., Mann, J.R., and Singer-Sam, J. (1994) Quantitative RT-PCR assays show Xist RNA levels are low in mouse female adult tissue, embryos, and embryoid bodies. Development 120:3529-3535

Pfeifer, G.P. (1995) Strategies to analyze DNA and nucleoprotein structure in vivo. in DNA and Nucleoprotein Structure in vivo. eds. H.P. Saluz and K. Wiebauer. R.G. Landes Company

Mann, J.R., Szabo, P.E., Reed, M.R., and Singer-Sam, J. (2000). "Methylated DNA sequences in genomic imprinting" in Critical Reviews in Eukaryotic Gene Expression (Eds. Stein, G.S., Stein, J.L., and Lian, J.B.)

Patents

16 patents

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