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University of California • Berkeley

Program in the History of the Biological Sciences and Biotechnology

Daniel G. Yansura

SENIOR SCIENTIST AT GENENTECH

With an Introduction by
Paul Godowski

Interviews Conducted by
Sally Smith Hughes
in 2001 and 2002

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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Daniel Yansura, 2000

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BIOTECHNOLOGY SERIES HISTORY--Sally Smith Hughes, Ph.D.

Genesis of the Program in the History of the Biological Sciences and Biotechnology

In 1996 The Bancroft Library launched the Program in the History of the Biological Sciences and Biotechnology. 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, Bancroft had no coordinated program to document the industry or its origins in academic biology.

When Charles Faulhaber arrived in 1995 as Bancroft's 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 and the pioneers 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 and archival 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 plans to safeguard the archives of individuals and businesses making significant contributions to molecular biology and biotechnology. 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 the History of the Biological Sciences and Biotechnology was given great impetus by Genentech's generous pledge to support documentation of the biotechnology industry.

Thanks to these generous gifts, Bancroft has been 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 publicity through ROHO news releases and announcements in scientific, medical, and historical journals and newsletters and via the ROHO and UCSF Library Web pages.

Oral history as a historical technique has been faulted for its reliance on the vagaries of memory, its distance from the events discussed, and its subjectivity. All three criticisms are valid; hence the necessity for using oral history documents in conjunction with other sources in order to reach a reasonable historical interpretation.¹ Yet these acknowledged weaknesses of oral history, particularly its subjectivity, are also its strength. Often individual perspectives provide information unobtainable through more traditional sources. Oral history in skillful hands provides the context in which events occur--the social, political, economic, and institutional forces which shape the course of events. It also places a personal face on history which not only enlivens past events but also helps to explain how individuals affect historical developments.

Emerging Themes

Although the oral history program is still in its initial phase, several themes are emerging. One is "technology transfer," the complicated process by which scientific discovery moves from the university laboratory to industry where it contributes to the manufacture of commercial products. The oral histories show that this trajectory is seldom a linear process, but rather is influenced by institutional and personal relationships, financial and political climate, and so on.

Another theme is the importance of personality in the conduct of science and business. These oral histories testify to the fact that who you are, what you have and have not achieved, whom you know, and how you relate have repercussions for the success or failure of an enterprise, whether scientific or commercial. Oral history is probably better than any other methodology for documenting these personal dimensions of history. Its vivid descriptions of personalities and events not only make history vital and engaging, but also contribute to an understanding of why circumstances occurred in the manner they did.

Molecular biology and biotechnology are fields with high scientific and commercial stakes. As one might expect, the oral histories reveal the complex interweaving of scientific, business, social, and personal factors shaping these fields. The expectation is that the oral histories will serve as fertile ground for research by present and future scholars interested in any number of different aspects of this rich and fascinating history.

Location of the Oral Histories

Copies of the oral histories are available at the Bancroft, UCSF, and UCLA libraries. They also may be purchased at cost through the Regional Oral History Office. Some of the oral histories, with more to come, are available on The Bancroft Library's History of the Biological Sciences and Biotechnology Website: <http://bancroft.berkeley.edu/Biotech/>.

Sally Smith Hughes, Ph.D.
Historian of Science

Regional Oral History Office
The Bancroft Library
University of California, Berkeley
October 2002

¹The three criticisms leveled at oral history also apply in many cases to other types of documentary sources.

ORAL HISTORIES ON BIOTECHNOLOGY

Program in the History of the Biological Sciences and Biotechnology

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

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

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

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

"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

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

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

Oral histories in process:

Brook Byers

Stanley N. Cohen

Chiron Corporation

Roberto Crea

David V. Goeddel

Herbert Heyneker

Irving Johnson

Arthur Levinson

G. Kirk Raab

William J. Rutter, Volume II

Axel Ullrich

Keith R. Yamamoto

INTRODUCTION by Paul Godowski

I was well aware of the pioneering work that Dan Yansura had performed prior to my arrival at Genentech in 1988. In fact, it was this very work that had first piqued my interest in this fledgling biotechnology company. When I was introduced to Dan, he didn't fit my naïve image of what a "biotech pioneer" should be. While some of my other "scientific heroes" exuded an air of confidence that sometimes crossed the border into healthy arrogance, Dan was quiet and genuine. While others had parlayed their success at the bench into "bigger and better things" Dan seemed quite happy working in the same labs where he started at Genentech in 1978.

Having been a colleague of Dan for nearly 15 years, I think I've come to understand why Dan was able to achieve the scientific successes that he has. Above all, he has a desire to accomplish things in the lab that others have not. I once suggested to Dan that he drop a project that I thought was too difficult. Dan sternly, told me that he "was going to get this project done in six months, no matter what anyone else thought." He was right, and I still have a chill that runs up my spine when I recall the ferocious determination that led to that ultimatum. At that moment, I also understood why Dave Goeddel's nickname for Dan was "Dynamite." Dan keeps the passion that he feels about his science to himself; on occasion, when others are allowed to see this passion, it can best be described as explosive. Most importantly, Dan challenges himself to accomplish the impossible simply to satisfy his own standards of excellence, and not because he seeks the adulation of others.

Dan has seen biotech grow from a single company with a handful of employees, half a lab, and a single office to a multi-billion dollar industry employing tens of thousands of people. Through it all, Dan has avoided the hype and continued to focus his extraordinary scientific talent on tackling challenging projects at the bench.

If you are lucky enough to meet Dan, don't let his quiet demeanor fool you. Underneath that kind and gentle exterior lies the heart of a lion.

Paul Godowski, Ph.D.
Senior Scientist, Molecular Biology
Genentech, Inc.

South San Francisco, CA
December 20, 2002

INTERVIEW HISTORY--Daniel G. Yansura

Daniel Yansura arrived at Genentech in June of 1978, only a few months after it had acquired a physical location in South San Francisco, and has remained there ever since. His long history with the company, currently in the position of Senior Scientist, and participation in key research projects make him an apt subject for interviews on Genentech. Although a chemist by education, like fellow graduate student David Goeddel he had received a solid background in the new genetic technologies, especially DNA synthesis, in Marvin Caruthers's lab at the University of Colorado.

Early in 1978, Goeddel had joined Genentech as a staff scientist and soon was trying to recruit Yansura to the young genetic engineering company. Dan did not take much convincing. Like Goeddel, he was anxious to apply recombinant DNA in the production of commercial products. He joined Genentech with the proviso that he would not have to work in DNA synthesis, which he had found repetitious and boring in the Colorado laboratory. He got his wish and more. He immediately became engaged in Genentech's all-out effort to clone and express the gene for human insulin, the hottest race in genetic engineering at the time. His account of the insulin project and the concomitant project on human growth hormone contributes yet another dimension to the Rashomon effect that the oral histories in this series cumulatively provide of the earliest years of commercial biotechnology in the Bay Area.

Where Yansura contributes entirely new information is in regard to Genentech's work on foot and mouth disease, a major scourge of cattle. He was a scientist on this project at a time when Genentech was willing to work in a variety of fields, not just in human pharmaceuticals where its focus is today. Yansura tells of his work on a recombinant approach to a vaccine, at Genentech and also for brief periods on Plum Island, the government's high-containment facility off the coast of Long Island, New York, where all research with the highly dangerous virus had to be conducted. In 1981, the U.S. Department of Agriculture triumphantly announced "a breakthrough in genetic engineering to produce a vaccine against the virus of foot and mouth disease, one of the world's most serious animal diseases." Genentech followed with its own publicity release. It was research for which Yansura and colleagues received an award warranting an editorial in the journal *Science*.¹

Oral History Process

Four interviews were conducted in Yansura's office overlooking the bay in one of the research buildings at Genentech. Reflecting the factual perspective of a scientist, he answered questions in a straightforward and amiable fashion. As elsewhere in this series, the chronology of his narrative was disrupted by Genentech's request to postpone discussion of the growth hormone work until the City of Hope trial had ended. It appears therefore in the last interview. As with all interviews in this series supported by Genentech, the transcripts were sent to its legal department for review solely for legal matters. As is true in every case to date, no changes were requested.

Sally Smith Hughes, Ph.D.
Historian of Science

Regional Oral History Office
The Bancroft Library
University of California, Berkeley
November 2002

¹ "Block Announces Production of Foot and Mouth Disease Vaccine," United States Department of Agriculture, June 18, 1981; Genentech, Inc. [n.d.]; Philip H. Abelson, "Foot-and-Mouth Disease Vaccines," *Science* 1982, 218 (December 17).

Regional Oral History Office
Room 486 The Bancroft Library

University of California
Berkeley, California 94720

BIOGRAPHICAL INFORMATION

(Please write clearly. Use black ink.)

Your full name Daniel George Yansura
 Date of birth Aug. 15, 1950 Birthplace Detroit Michigan
 Father's full name George Yansura
 Occupation Auto-factory worker Birthplace Barnesboro PA
 Mother's full name Bernice Paluch
 Occupation House wife Birthplace Detroit Michigan
 Your spouse/partner Patricia Rose Tanttala
 Occupation Math tutor Birthplace Seattle Washington
 Your children Julia Rose Yansura
 Where did you grow up? Detroit Michigan
 Present community Pacific CA
 Education B.S. Wayne State University
M.S. University of Colorado
 Occupation(s) Scientist

Areas of expertise Protein expression in bacteria

Other interests or activities Propagating and collecting exotic ferns,
hiking, camping, travelling

Organizations in which you are active American Fern Society
Los Angeles International Fern Society

SIGNATURE Daniel Yansura DATE: 9-15-02

INTERVIEW WITH DANIEL G. YANSURA, PH.D.

I FAMILY BACKGROUND AND EDUCATION

[Interview 1: October 1, 2001]##¹

Family Life and Environment

- Hughes: Let's start with where you were born and educated, and a little bit about your family life.
- Yansura: I was born in Detroit, Michigan. My dad was a factory worker. The only thing he wanted from his kids was for us to go to college, so we all went to college.
- Hughes: How many children were there?
- Yansura: Three children. I am the youngest.
- Hughes: What would you say about family dynamics?
- Yansura: I was the one that was sort of left out. Being the last child, most attention went to my sisters, so I just groped along as best as I could. Living in Detroit was not a very great experience, but that's the way it was. By the time I was eighteen or so, I knew I wanted to leave and pursue a career somewhere else.
- Hughes: Why did you want to leave?
- Yansura: Detroit is a factory town. The values of the people there were different from mine. People are interested in their cars, and there's not much intellectual life. It just wasn't the kind of place I wanted to end up.
- Hughes: Did you consider yourself a budding intellectual?

¹## This symbol indicates that a tape or tape segment has begun or ended. A guide to the tapes follows the transcript.

- Yansura: I didn't think of it that way. I realized at the time that was just where my interests were. My dad certainly wanted all of us to go to college. My mother never finished high school, so we were trying to beat my dad. He was trying to push us beyond his experience in life.
- Hughes: Did your mother back him up in his college ambitions for the children?
- Yansura: She did, yes, but she was a little more traditional; she just sort of followed my dad.

Education

Interest in Chemistry

- Hughes: Did you do well in school?
- Yansura: Yes, I did reasonably well in school and had a normal childhood.
- Hughes: Did you have any particular interest in science early on?
- Yansura: Yes, in grade school I became interested in chemistry. We didn't have much science in grade school back then, but we did have one science class. People brought in things--show-and-tell for science. One boy brought in a series of bottles with liquids in them, with all these different chemicals. He got these from his dad who worked at an oil company, and these were different organic compounds, like pentane, octane. They all looked the same; they were all colorless liquids in bottles. I was just fascinated for some reason. I started to become interested in chemistry, and there was a chemistry book in our limited library at home, or bookshelf I should say. It was my sister's high school chemistry book. So I started reading that and I just became fascinated with chemistry.
- Hughes: What age were you?
- Yansura: I was probably in sixth or seventh grade. I would go to the basement and do chemistry with whatever I could find around the house. I had a wonderful time doing that for a while.
- Hughes: Did you have a chemistry set or was this make-do?
- Yansura: This was make-do.
- Hughes: There wasn't money for things like that?
- Yansura: My dad was fairly tight with money. It was probably just as well because it forced me to read labels on household chemicals, like Drano is sodium hydroxide, and so forth. I ended up reading this chemistry book and playing with all of the chemicals I could find around

the house and doing the best I could. I had electrolysis going to make different chemicals. I was making hydrogen and so forth. This was all fascinating.

High School

- Yansura: I went to a high school called Cass Technical High School in Detroit. It's a magnet school. They had a curriculum in biology and chemistry.
- Hughes: Was that why you wanted to go there?
- Yansura: It wasn't exactly. My sister had gone there about two years before I did, and she said it was just such a wonderful school. It was a magnet school through the whole metropolitan area, and people not only from Detroit but from the suburbs would come in, maybe driving an hour or so, just to get to the school.
- Hughes: Was there an admissions exam?
- Yansura: There wasn't an exam, but you did have to get their okay. So you had to somehow give them your grades. They only took who they wanted.
- Hughes: What did the school do for your interest in chemistry?
- Yansura: It was a wonderful experience. Everyone was dedicated to one little area: you could go into the arts or engineering or electrical or whatever, and I went into chemistry and biology. I had three years of chemistry there.
- Hughes: Which was unusual for high school.
- Yansura: Which was unusual.
- Hughes: How far did that carry you?
- Yansura: We had one year of general chemistry, a year of analytical chemistry (including both qualitative and quantitative chemical analysis), and then we had organic chemistry. In college I skipped my first year of chemistry.
- Hughes: That's a lot of chemistry to come to college with.
- Yansura: Yes. It is something I wish my daughter had. I think that's what all kids should have available.

Undergraduate, Wayne State University, 1968-1973

Hughes: Why did you go to Wayne State?

Yansura: I realized, not having a whole lot of money, I had to go somewhere reasonably cheap in the state of Michigan. Wayne State was convenient.

Hughes: Is it in Detroit?

Yansura: It's right in Detroit.

Hughes: And you lived at home?

Yansura: I lived at home because the surrounding areas of the campus were like inner-city ghettos, so nobody wanted to live there. There was a nice campus, but you just wouldn't want to live near there.

Biochemistry Research

Hughes: What did you do at Wayne State? Were you on a chemistry tract?

Yansura: Yes. I didn't decide right away. I had a few other interests. I didn't want to commit to it, and yet I immediately started following the courses that were required for a chemistry degree because I didn't want to get behind. I eventually got a bachelor of science degree, in 1973.

Hughes: Did you have a special relationship with any of your professors?

Yansura: I didn't have any particular relationship with any of the professors in the chemistry department, although I started a part-time job my second year in college working in a laboratory at the medical school. There I worked for Professor Joseph Pfiffner in the physiology department. The project was extracting vitamin B-12 analogs or corrinoids from photosynthetic bacteria, with the idea of finding novel and potentially pharmacologically useful compounds.

Hughes: Did that engage you?

Yansura: Yes, that engaged me even more. It pushed me a little from chemistry into biochemistry. As an undergrad my interests were going towards organic chemistry. I still have a fondness for organic chemistry. But working in this laboratory at the medical school with real live things--purple photosynthetic bacteria called *Chromatium*. We were extracting things from them, and it was just fascinating to me. I spent a lot of hours there.

Hughes: What was the fascination?

- Yansura: It made me realize biochemistry was an offshoot of organic chemistry. I had this fascination with organic chemistry way back in grade school when this student brought in these little vials of colorless liquids that all looked the same. So I had been interested in organic chemistry all this time, and now all of a sudden I could see that biochemistry was an upper layer on top of that.
- Hughes: It wasn't that biochemistry was related to living forms?
- Yansura: At the time I started to see living things and nonliving things sort of as a continuum. Even to this day I don't really see this break of living things and nonliving things; it's all organic chemistry to me.
- Hughes: That's an interesting perspective considering that you're heavily into the biological end of things now.
- Yansura: Yes, but I look at it as organic chemistry in a lot of ways.
- Hughes: How do you think that shapes what you do in the lab?
- Yansura: I think it makes me less biased about what's going on. Instead of saying that this is some biological life form process, I say that this is chemistry and I need to understand it. It's just chemistry; that's all it is. That's my perspective, and it leads sometimes to a friction or a different dialogue with scientists who have views that living forms are something special.
- Hughes: Vitalism is alive and well.
- Yansura: I hate it because I view vitalism as more religion than science.
- Hughes: Did you have a relationship with Professor Pfiffner?
- Yansura: He was just the boss. Most of the time he was working in his office, so I didn't work that closely with him. He was certainly a nice guy, and I respected him, but it was more the ability that I had to work in his lab and expand my interests.
- Hughes: Did he have graduate students?
- Yansura: He did not have graduate students. He had postdocs.
- Hughes: Why no graduate students? Is that significant?
- Yansura: It might have been significant, although at the time I didn't notice what the significance was. There were a number of other professors on the floor, some of them did have graduate students and some just had postdocs. I just don't know. He was an older professor, so by the time I left he was already going into retirement.

Mentor

- Hughes: Were you mentored in any way by the postdocs in his lab?
- Yansura: Yes, there was one postdoc. I'll have to take a guess at his name; I think it was Dr. Koppenhagen. He was a German postdoc. He let me take over some of his work. I was chosen out of the pool of other undergraduates that were working there.
- Hughes: He saw your potential.
- Yansura: He must have; I don't know why he chose me. So he did have some influence on me. This was the first time that I could talk easily and comfortably with a scientist about biochemistry.

Graduate School, University of Colorado, 1974-1976

Selecting the School

- Hughes: Is the next step the University of Colorado?
- Yansura: Yes.
- Hughes: Tell me how you happened to go there.
- Yansura: I had always wanted to leave Detroit. As I said before, the value system there was different from mine. I was interested in more intellectual and cultural things. I decided this was the chance; I was going to leave Michigan once and for all. I applied to a couple of schools, and one of them was the University of Colorado in Boulder.
- Hughes: What were your criteria?
- Yansura: I wanted to pick a school that was reasonably good in chemistry; in other words, chemistry was highly established there and respected. I would go to *Chemical Engineering News*. Every year they publish where all the universities stand in chemistry, based on number of grants or whatever. University of Colorado was a reasonably respected school. There was one graduate student I can remember in the place where I was working then, which was the department of physiology in the medical school. He said that he had been there and it was a beautiful campus, just a wonderful place to live. By then I was excited about going.

Of course the University of Colorado is right next to the mountains. It is a beautiful campus. Colorado's a beautiful place to live in a lot of ways. I had come from a place where everything was dirty, a car-based factory town. I had no idea what to expect out there. Mountains didn't mean a whole lot to me then because I had never really seen any

mountains. But by the time I left, the outdoor life had really sunk into me. From then on I've always been an outdoors type of person.

David Goeddel, Graduate Student, Division of Biochemistry, Department of Chemistry

- Yansura: The relationship between Dave Goeddel and myself was important, and the fact that Dave had come from California influenced me. He had lots of mountaineering experience; he was a climber, a camper, a backpacker, a fisherman, whatever. He could do all that. The first few years in graduate school we would go in the mountains, fishing and more fishing. That got me into the mountains for the first time and expanded that part of my life.
- Hughes: Could you keep up with him?
- Yansura: Nobody ever tried to keep up with Dave Goeddel.
- Hughes: [laughter] Is that a blanket statement?
- Yansura: That's the statement that I make out of wisdom. Dave is somebody I really respect and I like, but you don't really want to keep up with him.
- Hughes: That would be an all-consuming ambition, wouldn't it?
- Yansura: Yes, it would. It's no way to live your life. [laughter]
- Hughes: Give me a feeling of what those mountain trips were like. You went off on a weekend?
- Yansura: Usually. Because we were in graduate school then we didn't have a whole lot of time to take off. We would usually go for an over-night trip. Sometimes in the winter we would just go fishing for the day. Dave was an avid fisherman, and he still is. Fishing was okay with me, so I would go along. But I was definitely more interested in just being outdoors and seeing things I had never seen growing in Detroit. That was a very positive experience.
- Hughes: So were these physically exerting trips? Did you backpack long distances and climb mountains?
- Yansura: No, we would take the car, park it somewhere, and go up some mountain stream. Then maybe we would camp there later on.
- Hughes: Just the two of you?
- Yansura: It started off maybe the two of us and then eventually we had the whole lab going.

Marvin Caruthers's Laboratory

Selecting the Laboratory

- Hughes: You ended up in Marvin Caruthers's lab. How did that happen?
- Yansura: When you first get to the University of Colorado they ask you to talk to all the professors in the department. It was the biochemistry division of the chemistry department. They ask you to talk to all the professors and see what they're doing in their research, and then you decide whom you want to work with. They give you a list of what all the professors are working on before you even arrive so you have some idea. I had already picked out two or maybe three professors to work with, and one of these was Marv Caruthers.

Synthetic DNA

- Yansura: At the time, Marv was working on synthetic DNA, and I just had a hunch that this was going to be really important and was going to break out, somehow, into the biotech industry. Which it did. I had at the back of my mind that there was going to be a payoff here.
- Hughes: Meaning commercially?
- Yansura: I wasn't thinking of it like commercial or personal finance for myself. I just thought synthetic DNA was going to be a new field that was going to expand. It was very early in that field, and I thought this would be a really fun thing to do.
- Hughes: Had you paid any particular attention to the work that was being done on DNA? Had that been emphasized in your previous studies?
- Yansura: I had read the book--I'm trying to think of what the name of it was now--that was read by Biochemistry 101 students.
- Hughes: Watson's book?
- Yansura: I think it was Watson's book about the gene.² It was a very readable book that explained the very simple ideas of the gene at the time, control of genes and development. I had read it before I had gone to the University of Colorado and found it very interesting. I had seen what Marv Caruthers was doing with synthetic DNA. I had a pretty strong feeling that this was really going to take off.

²James D. Watson, *Molecular Biology of the Gene*, Menlo Park, CA: W.A. Benjamin, Inc., 1st edition, 1965.

Hughes: Why did you think that?

Yansura: It was so obvious.

Hughes: It wasn't obvious to a lot of people, was it? You're talking about around 1973?

Yansura: Yes, around 1973. I didn't know what other people were thinking, but to me it was so obvious that this was going to really break open.

Hughes: Was it just a matter of meeting Dr. Caruthers and signing on? Was it that easy?

Yansura: No, there was luck in the whole thing. At the time, Marv Caruthers was making synthetic DNA of particular gene control regions. So he was interested in looking at the biology associated with the DNA. His career had evolved out of coming up with the ways to make synthetic DNA, which was not a trivial thing. My interests and his were going towards using synthetic DNA as a technology with which you can do biology on. That's sort of what we did.

Dave Goeddel and I worked on lac operator DNA. We made a synthetic DNA operator and studied the binding and looked at mutations.

Hughes: Dave preceded you in Caruthers's laboratory?

Yansura: No, we arrived essentially at the same time.

Hughes: And you're about the same age, aren't you?

Yansura: Yes, approximately.

Hughes: So you went through the graduate program shoulder to shoulder?

Yansura: Yes, we started off from the same place. I believe we were Marv Caruthers's first graduate students.

Hughes: Caruthers was a young man?

Yansura: Yes, this was his first real job, as an assistant professor. He was starting up a lab. He had about four or five potential projects to work on, and Dave and I both decided to work on the same one.

Hughes: The lac operator?

Yansura: The lac operator.

Hughes: Where had Caruthers developed an interest in synthetic DNA?

Yansura: Marv Caruthers had been a postdoc in Gobind Khorana's lab. Khorana won the Nobel Prize for making a synthetic gene for a bacterial tRNA or transfer RNA.

Hughes: Had he already gotten the prize when you got to Colorado?

Yansura: Yes.

Hughes: Was that a sign that synthetic DNA was a hot area?

Yansura: You can say that, but people win Nobel Prizes in different areas every year. The research that Khorana did was in itself not that monumental. He made a synthetic DNA, a gene for a tRNA; you can't do anything with it. We always thought that that in itself wasn't interesting. What was really of value, was that he had developed a way to make synthetic DNA.

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Yansura: Khorana had come up with the technology to show that you could make small pieces of synthetic DNA and ligate them together to make bigger genes, and that you could make at least a modestly sized genes back then. That was the value.

Hughes: Was the technology that you learned when you went to Caruthers's lab close to what Khorana had been using?

Yansura: Yes, we started off with the same technology. We made a synthetic lac operator that way. A few years later Marv had advanced the synthetic DNA technology and he had come up with a new way--I shouldn't say simpler--but a better way to make it. That is what also really propelled the biotech industry.

Hughes: Caruthers did that on his own or were you involved?

Yansura: I wasn't involved. There was another graduate student, Mark Matteucci.

Hughes: Didn't he come to Genentech?

Yansura: He was here for a while also. He started a little bit later than Dave Goeddel and myself. Mark Matteucci was more of an organic chemist, and so when he worked in Marv's lab they came up with a new way to make DNA. They patented this new method, and apparently Marv is quite wealthy from this new invention.

Hughes: Did Caruthers call himself a biochemist or a chemist or something else?

Yansura: He was really a chemist at heart.

Patenting in Academic Biology

Hughes: Was patenting in chemistry unusual?

- Yansura: Yes, it was fairly unusual. Maybe not so much in private labs, commercial labs, but certainly in the university it was not that common.
- Hughes: Was it talked about that Caruthers had patented this work?
- Yansura: At the time I was there it hadn't been patented yet. There was another professor there that had a patent on some chemical compound. He was studying some enzyme that interacted with nerve gas, and he came up with an inhibitor of the nerve gas. He had a patent on it, and everybody would talk about it like it was something really cool.
- Hughes: Oh, cool?
- Yansura: Everyone had publications, but this guy had a patent.
- Hughes: So that was a plus?
- Yansura: That was a plus.
- Hughes: As you probably remember, when the biotech industry came along later on, some people were dubious about the propriety of patenting, in biology anyway.
- Yansura: Yes, and that changed.
- Hughes: Why was there a problem when patenting began to be done in the biological area?
- Yansura: Some felt that academic science should be free from any legal constraints. They were just purists. Then there were the more pragmatic people who realized that if you don't patent it, someone else might. The way patents work is that generally any researcher can use whatever's patented. Generally, the patent only inhibits commercial development of some idea. Patenting really serves everybody's purpose, I think. And certainly in graduate school we didn't think there was anything wrong about patenting some idea. On the other hand, we thought it was definitely quite ingenious. If you could do it, why not?
- Hughes: Did you patent while you were at the University of Colorado?
- Yansura: No, and most of the stuff we were doing was not patentable.
- Hughes: It was basic science?
- Yansura: Yes, it was basic science.

Research and Pranks with David Goeddel

- Hughes: Describe how you and Dave Goeddel worked together in the lab at the University of Colorado.

Yansura: You may know about Dave Goeddel. He was a very--I wouldn't say aggressive--but he's very determined. When he goes after something, he goes after something. He doesn't attempt to do well with people who are slacking off or who are not real motivated along with him. I personally got along with Dave very well.

Hughes: Because you have those characteristics?

Yansura: Yes, in some ways. I take my work very seriously, and I think Dave respected me for what I could do. At the same time I wasn't as much of a diehard as Dave Goeddel.

Hughes: Few people are.

Yansura: That's true.

Hughes: Did that sometimes make for tensions?

Yansura: Yes, there were tensions in the lab.

Hughes: Do you want to say more?

Yansura: Sure. Marv Caruthers just had a reunion of all of his students. He won the distinguished professor award at the University of Colorado, and they threw him a symposium. They asked all of his former students to come back for it, and we went back this last summer. We got to see all these old friends we haven't seen for years.

The stories are that Dave Goeddel had some frictions with some of the other members of the lab. Generally, I would be on Dave's side because we were a team; we were working on the same project, and in general I agreed with him on these little frictions between people. I didn't agree with the way he was approaching it, which was intense. We eventually got into some trouble with Marv Caruthers over this, and he would call us into his office.

Hughes: What was Caruthers trying to achieve with you two?

Yansura: He was trying to control us in some way. Obviously, he had to keep harmony in the lab; you can't knock him for that. And certainly we were the obvious instigators of trouble. We did a lot of pranks. Most of the time we were making synthetic DNA, and it's really boring. I wanted to go beyond making synthetic DNA, and Dave Goeddel didn't want to be making synthetic DNA all the time either; he was seeing beyond that, like I was. It was really boring, so we had to do something to keep from going crazy.

Hughes: So what did you do?

Yansura: We had all sorts of pranks. For example, we had a summer student who was a pre-med. Everybody hated pre-meds at the time.

Hughes: Why?

Yansura: Pre-meds are undergraduates who want to get into medical school at any cost, so that's all they care about. Most science students tend not to like pre-meds so much.

We had this pre-med coming in who I think was an undergraduate at Yale, and he was very elitist. You could just see his time was coming. David put together this fishing trip, and we convinced this pre-med--his name was Eli--to come along with us. At the very start of the hike Dave Goeddel got out a heavy iron frying pan--the heaviest thing he could think of--and he told Eli, "Someone has to carry this pan because we need to fry the fish once we get up there." So he put it in this guy's pack, and Eli struggled up into the mountains with this twenty-thirty pound metal pan in his pack.

So we had a lot of pranks to get over the boredom. Eventually we ended up accomplishing things in the lab besides that. We were pretty much all strong performers, the first group of students.

Hughes: How much interaction did you have with Caruthers?

Yansura: We would have weekly lab meetings, and he would come by the lab once or twice a week.

Hughes: Otherwise were you pretty much left on your own to pursue your projects?

Yansura: For the most part we had our sight set on what we were going to accomplish, and we would decide ahead of time what we were going to do. It might take months and months to carry that out.

Research on the lac Operator

Hughes: What did you accomplish in terms of the basic science of the lac operator?

Yansura: We used Khorana's method of making synthetic DNA, so we hadn't done anything new there, and that wasn't really what we wanted to do. We wanted to explore the interaction between a protein, the lac repressor, and DNA. The synthetic DNA that we made was ligated or glued together to make a 26-base pair duplex. We had the sequence memorized after a while. In fact, I could still probably say it because it's so ingrained, and we were making this stuff so slowly.

Eventually we mapped some of the contacts between the repressor and the DNA. Some of these were the expected types of contacts, such as amino groups in the grooves of the operator DNA presumably forming some hydrogen bond with the repressor. One of the interactions though was with the methyl group on thymine which we thought was very unusual. I think it was the first time that anybody had seen anything like that. Knowledge of the interactions between DNA and protein at that time was very, very primitive. Wally Gilbert had sequenced the operator, and there were certainly a lot of studies done on the interactions, by looking at mutants for example. We had the ability to change one

nucleotide at a time, very specifically one small part of that nucleotide, look at the interaction, and go from there.

Marvin Caruthers

- Hughes: What was Caruthers like as a personality?
- Yansura: Everybody loved Marv Caruthers. He was sort of the father figure. It's hard to say anything bad about Marv. We always made jokes about how he dressed.
- Hughes: Which was how?
- Yansura: The classic is, he wore an orange shirt and a purple tie. We still make fun of him to this day about that. We later had orange T-shirts made for the lab with purple ties printed on the front, and on the back it said "Marv's Morons". He was very likeable. You could talk to him very easily.
- Hughes: You felt that he was engaged in what you were doing? He wasn't a distant lab chief?
- Yansura: He was definitely engaged. He was trying to earn tenure, and you have to start off running and even then be lucky. He was very serious about that. I found by going to this reunion that a lot of his later students said that he wasn't around much. He was later on the board of directors for Amgen, and he was nominated to the National Academy of Sciences. So he was moving around a lot more and less available to the people in his lab.
- Hughes: You had quite a number of publications as a graduate student. Maybe more than the average?
- Yansura: Yes, compared to a lot of graduate students.
- Hughes: Why?
- Yansura: Marv was very interested in publishing, and he was just on it. I'm sure that had to do with the fact that he wanted to earn his tenure, and he had to be pretty driven to do that. The second thing is that we got a fair number of results worth publishing.
- Hughes: Was synthetic DNA looked upon as a hot area?
- Yansura: No. I told you earlier that I saw it as a very early stage of using synthetic DNA to do genetic engineering. But a lot of other scientists didn't feel that way. In fact, we got a few negative replies from Larry Gold who was a professor in the Molecular, Cellular and Developmental Biology Department at the University of Colorado. He would make fun of us trying to study biology by making synthetic DNA. His comments were, "What can you do with this stuff? It doesn't show you any new biology or new ideas." So he was definitely negative on it.

- Hughes: You had been seeking him out, hoping for a collaboration?
- Yansura: No, Dave and I had a course with him. Larry Gold was well respected; he was very smart. I respect him for a lot of reasons, but he was definitely negative on synthetic DNA. He was very successful at using genetics to study his systems. He worked a lot in *E. coli* genetics and was very successful at using genetics with some biochemistry. He didn't see the usefulness of making synthetic DNA to use as a starting point of some experiment.
- Hughes: Had you thought of going on to doctoral work?
- Yansura: Yes, I started off wanting to get a Ph.D. One of the things that held me up was a fear of talking in public.
- Hughes: Having to defend your thesis?
- Yansura: Not so much that, it was the constant talks, the seminars. That's part of the job, and I couldn't face that at the time so I decided I better do something else.
- Hughes: What happened between your master's degree [1976] and going to Genentech?
- Yansura: I stayed on at Marv Caruthers's lab for a year or two after I had gotten my master's degree. He asked me to stay on and do more work along the same lines. He had a number of grants back then. So I decided it was a good idea. I loved living in Boulder, Colorado. I liked the work. I didn't see any opportunity out there yet, because there wasn't really much demand for scientific talent in this new area.
- Hughes: The new area being genetic engineering?
- Yansura: Yes.

Early Stages of Recombinant DNA

- Hughes: By then had you learned recombinant DNA technology?
- Yansura: In Marv Caruthers's lab we had done a number of the basic steps. We had worked with synthetic DNA all the time and had been ligating it together. We had barely gotten to the point where we were putting it in plasmids, although we didn't do that work ourselves; we had to collaborate with a professor called John Sadler at the University of Colorado Medical Center in Denver.
- Hughes: And he was constructing plasmid vectors?
- Yansura: Yes, he knew how to do the transformation and the follow up. And in Marv's lab we knew

how to ligate everything together, so we collaborated on that. Between the two of our groups we had done the first steps in genetic engineering, putting DNA into plasmids and replicating them, and so forth.

Hughes: Who else was doing that kind of work in 1977, '78?

Yansura: There were other groups making synthetic DNA. There was at least one other group that had made the same lac operator DNA that we had made, so there was competition there. So we were always worried that we were going to get scooped.

Hughes: Did you ever?

Yansura: Yes, in some ways we did, in some ways we didn't, and I think that's true of biotechnology as a whole. You see, sometimes another group appears to beat you, but it's not always that clear, and at that time all we saw was a publication we did or didn't get. Things are much more complicated now in the biotech industry. One group may publish first while another group wins patent coverage.

There was another group headed up by Ray Wu, I believe it was. They had made the synthetic operator and eventually cloned it. So we weren't the only ones in the field, but it was still relatively rare.

Hughes: You were cloning as well?

Yansura: Just at the very minimal stage of putting our lac operator into a plasmid, or ligating it into a plasmid.

II GENENTECH¹

A Job Offer and Then Marriage

Hughes: What month did you arrive at Genentech?

Yansura: I started at Genentech in June 1978.

Hughes: Had you married by this time?

Yansura: No, I was not married yet. My last year in Boulder, Colorado I met Patricia Tanttilla, and she came out here to San Francisco with me. We got married a few years later. We had just started our romance in Boulder at the time I had gotten a call from Dave Goeddel that he had a job opening here at Genentech.

Hughes: That was in 1978?

Yansura: Yes. I knew it was a good opportunity; I'd better do it. I didn't think about what would happen if the job or romance didn't work out. It was a period in my life when I was willing to take risks.

Hughes: Dave Goeddel went to the Stanford Research Institute in 1977 and arrived at Genentech early in 1978. He hadn't been there very long when you got there, had he?

Yansura: No, he hadn't. He joined up with Genentech maybe in March, and I came in June.

Hughes: Dave Goeddel asked you to come, and that's all it took? What was the process?

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Yansura: He told me that he was working for this brand new company that was trying to make human insulin by recombinant means. He told me about the synthetic DNA for making all

¹For better chronology, a portion of this section was moved from interview three.

the genes. Then he told me, "Well, we have to ligate them all together, and then we have to get the protein expressed." I thought that was a big jump.

Hughes: Because nobody had expressed a synthetic gene?

Yansura: Well, Boyer had done it with somatostatin a few years earlier [1977]. You knew it could be done, but it wasn't that clear that it was going to be easy. Somatostatin was a very small peptide attached behind this humongous *E. coli* protein called beta-galactosidase. *E. coli* would translate its own protein first, and then just keep going and translate somatostatin. But that doesn't mean it was going to be easy to express proteins. I thought it was really fascinating that we were going to not only make genes and put them together --because we had done that in Marv Caruthers's lab--but we were going to turn them into proteins. That was our job. And if we didn't accomplish that then there wouldn't be a job. I thought that was just terribly exciting.

Hughes: Did it make a difference that it was insulin, or would any protein have done for you?

Yansura: Any protein would have been exciting for me, but of course insulin is probably the most famous protein; everybody knows what it is. People don't know it's a protein a lot of times, but it's the most well-known protein.

Hughes: Did Dave Goeddel explain over the phone what he thought you might do on this project?

Yansura: No, he didn't. He said exactly what was going on, that synthetic DNA was being made, and that I wouldn't have to make synthetic DNA. I had enough of that. I realized back at the University of Colorado that I did not want to do DNA synthesis long term. The amount of solvents that we had to breathe was really not good for you. We would walk into the cold room and there would be spills of pyridine; it smells like old fish. You knew it wasn't good for you, and so I knew that I wasn't going to go that route. I wanted to go beyond DNA synthesis; I wanted to be able to use it and to manipulate bigger things. Then Dave told me about making protein and all that. The discussion was pretty general.

Hughes: Wasn't there some synthetic DNA capability at Genentech by then?

Yansura: It wasn't at Genentech at this time; it was down at the City of Hope. Roberto Crea was leading that effort although Keiichi Itakura was actually in charge.

Hughes: Why did you think you should accept the job at Genentech?

Yansura: There weren't any other jobs that involved the early stages of genetic engineering.

Hughes: And genetic engineering was what you wanted to do?

Yansura: Yes, I had predicted when I first started graduate school that this whole area was going to open up. Herbert [W.] Boyer and Stanley [N.] Cohen had published their papers on combining heterologous DNA or genes into plasmids and shown that you could transfer them, and so I knew that the whole area was going to open up. So when Dave called me and had this perfect job out there, exactly where I wanted to go, what else could I say?

- Hughes: Was there any discussion with Dave Goeddel over the phone about what you would be paid?
- Yansura: I think so, yes--\$18,000 a year. Back then, it sounded like a lot of money.
- Hughes: What would you have gotten in an academic position at that time?
- Yansura: At the University of Colorado, I was making \$11,000 or \$12,000 a year. I remember it was about a 50-percent boost in salary and so it seemed like a lot of money.
- Hughes: Did you have any concerns about this new company applying a new technology?
- Yansura: Yes. I was concerned that it might fail. Dave Goeddel made it pretty clear that if we did make insulin, and we were the first, then everything would be rosy. We would get a contract with Eli Lilly. But if we weren't first or we couldn't do it then it was clear we would all be looking for new jobs.
- Hughes: That didn't worry you?
- Yansura: Not at the time. We were all in our twenties, so it wasn't that big of a deal.
- Hughes: Was Dave convincing in making all this seem possible to accomplish?
- Yansura: Once you get to know Dave Goeddel, you realize that once he goes after something--in this case he would be going after insulin--that he does a pretty hard job at it. He does not like to lose. I felt we had as good a chance as any of succeeding.

Job Opportunities in Molecular Biology.

- Hughes: Did you feel that you had a marketable skill that not many other people had, namely your ability with synthetic DNA?
- Yansura: Well, there wasn't really a market, except at Genentech. It was all so new. There was a limited amount of synthetic DNA in the world at that time. There were only a few labs making it. It was a specialty.
- Hughes: If you hadn't gone to Genentech, you might have stayed at Colorado?
- Yansura: I could have stayed at Colorado because I was working for Marv Caruthers who was assistant professor at that time. Eventually he made it to full professor and did very, very well at the University of Colorado. But I would just be working in his lab, and I didn't see that as something I would do forever. It was not really a future.

- Hughes: What I'm trying to get at is how useful were the genetic technologies you had learned in getting you a position in academia or industry?
- Yansura: I always felt that there would be a new technology area opening up over synthetic DNA and the manipulation of the DNA to make proteins, and so forth. When Dave Goeddel offered me the job, there wasn't really a market for this skill yet, because it was too early.
- Hughes: But you had faith that it might come.
- Yansura: Yes, I knew it would come eventually.
- Hughes: By the late 1970s other companies were beginning to be formed. Biogen was pretty fast on the heels of Genentech, and Amgen as well.
- Yansura: Someone at Biogen, a former graduate student in Marv's lab named Eric Kawashima, wanted me to work for him at Biogen. I could have gone there, I suppose. They were stationed in Switzerland.
- Hughes: I suppose that Dave Goeddel, being a known entity--you'd worked very closely with him--must also have been a draw?
- Yansura: [pause] Dave Goeddel is sort of a mixed bag. Dave Goeddel is very successful, and when he goes after something there's a pretty good chance that's he's going to accomplish it, so it's good to be associated with him. He does have his rough sides. I had worked with him at the University of Colorado, so I was used to that, but for some people it was a hard thing to work for Dave Goeddel. He was so aggressive in wanting to accomplish this that if you slipped up on your part he would let you know.

Physical Layout and the Early Research Team

- Hughes: What did you find when you got here?
- Yansura: Not much. There were these long warehouse buildings with big, empty shelves. Building one still stands today. There were about ten different shipping companies in this big, long building, and Genentech had rented out a corner of it. So we had maybe ten percent of the building. You walked in and you saw a normal room, like an office with a little reception area. Then there was Bob Swanson's office in the corner. There was one lab that was about half put together. I think the lab benches were in, and I'm not sure if the shelves were in yet. It was still being finished. Once you went beyond that it was just a big, open warehouse room. You could see up two stories and there was nothing there. It was a challenge to think that you could accomplish anything here.
- Hughes: Did your heart sink?

- Yansura: No, because I had already made up my mind that this was going to work. I had come to the realization that if it didn't work it would still be okay. I had always wanted to live in San Francisco, so at the very least I would be able to live out here for a while and see what it was like.
- Hughes: Were you pretty confident that if this job failed that you could do something with your expertise in synthetic DNA?
- Yansura: No, there wasn't really any opportunity out there. It was putting all your eggs in one basket. Although I could do other things--biochemistry: I could isolate enzymes, and stuff like that. But it wasn't really my forte; it wasn't my expertise.
- Hughes: Because of the current lawsuit, we have to skip over the research on insulin that you got into immediately. Who was here at the time you arrived?
- Yansura: When I arrived there were only two scientists here, Dave Goeddel and Dennis Kleid.
- Hughes: What number employee are you?
- Yansura: Seven or eight.

DNA Sequencing

- Hughes: Did you do any sequencing at Colorado?
- Yansura: Yes, we had made synthetic DNA fragments that were to be ligated together to make the lac operator. Anytime you finished making your little segment of DNA you had to sequence it. We had a sequencing method that seems so primitive now, but we did use it. We had to verify the sequence of our fragments.
- Hughes: Was that another skill of yours that Dave Goeddel was anxious to acquire for Genentech?
- Yansura: No, the sequencing method that we used was by the time we left Colorado sort of obsolete. Allan Maxam and Wally Gilbert had come up with a new method of sequencing DNA that was for many years called Maxam-Gilbert sequencing. It was a very elegant method which you probably have heard about. That had just come out. In fact I have one of the original sequencing protocols sent to Marv Caruthers. It's from Allan Maxam who was one of the inventors.
- Hughes: [Reading from the protocol] It says, "Sorry to take so long getting these to you. It's a new version."
- Yansura: None of us had used the Maxam-Gilbert method at the University of Colorado.
- Hughes: What year is that document?

- Yansura: I don't think it says. Probably about 1977.
- Hughes: That sounds right.
- Yansura: Marv had given this protocol to us, but we had never used it in his lab, so I brought it here.
- Hughes: Did the City of Hope people use the Maxam-Gilbert method?
- Yansura: To sequence their synthetic DNA?
- Hughes: Yes.
- Yansura: No, I'm sure they didn't.
- Hughes: It was too early?
- Yansura: Yes, it was too early in the process. For shorter pieces you usually can't use that method. Using Maxam-Gilbert, you have to run your labeled DNA fragments on polyacrylamide gels, and you can't resolve the small fragments that you get with synthetic DNA. I'm not sure if they ever sequenced their synthetic DNA down at City of Hope, or if they counted on us to do it after it was cloned. Roberto Crea and the others involved published a paper on making the synthetic DNA for the insulin genes, and it was published along with our paper on the cloning and expression of it.
- Hughes: Roberto had the sequencing responsibility as well?
- Yansura: Well, Roberto was one of the key figures involved in making the synthetic DNA at the City of Hope. I'm not sure if he sequenced all the little fragments or not. Certainly it was standard; when we made the lac operator, we had to sequence it all in the lab. If you're not going to publish it you don't have to sequence it. You just take a chance, I guess.

Business Aspects: Initial Public Offering, Junior Stock, and Early Executives

- Hughes: About two years after you arrived, Genentech had its initial public offering. How aware were you that the IPO was in the offing? Was this a big event, even for the scientists?
- Yansura: Yes, it was a big event. We had all gotten shares of Genentech stock early on, and most of us had no idea--at least I didn't really know much about stock. When they first offered me stock, they said it was going to cost \$300, and I said, "Let me think about this overnight". I actually said that! Eventually we all bought what was called junior stock. When the company went public, all of a sudden you had a value to that stock. Before that it was worthless.
- Hughes: What was junior stock?

- Yansura: It was equivalent to regular common stock in all aspects except if the company went bankrupt, they would sell off all the assets - the equipment, and stuff like that--and that money would go to the holders of the common stock, whereas if you had junior stock you wouldn't get anything.
- Hughes: How much did Swanson tell you about the IPO before the actual Wall Street offering?
- Yansura: He would communicate with us what was going on. We would get little handouts in our mailbox. But pretty much he was handling all the business sides of the company, and he had a few other people in finance that would help him with that aspect.
- Hughes: Were they around in your lives on a daily basis?
- Yansura: Yes. The company was so small, you could not help seeing everybody in the company within the first hour.
- Hughes: People who weren't scientists wandered through your lab, for example?
- Yansura: We had a window in the lab, facing the hallway, and on the other side of the hall was Fred Middleton's office. He was vice president of finance. He had no idea what we were doing, except for the bigger picture.
- Hughes: And vice versa?
- Yansura: And vice versa. But we all got along well.
- Hughes: You were all young and starting off on a new thing.
- Yansura: Yes, we were all pretty much the same age. There was one older person, Brian Sheehan, an engineer. He was definitely of an older generation. He was very nice, and I liked him, but you could somehow tell. He was very wary of us and what we were doing. Eventually he got fired. Bob Swanson hired him to do the engineering aspects of growing up bacteria in these big fermenters so that we could produce human insulin and other proteins. He really didn't have the knowledge or the capability of doing that, and so he would sweep floors in the back.
- Hughes: He couldn't do the job for which he was hired. It wasn't that Genentech was too risky, or the rest of you were too young for him to be comfortable with?
- Yansura: In some ways it was uncomfortable. He seemed to be set for an older company with very traditional systems of management and science, and we scientists and Bob Swanson were from a different generation. So he didn't quite fit in, and there was a suspicion that you felt that he had about us.
- Hughes: Getting back to the IPO, what difference, if any, did it make once the company had gone public? Was that a factor in your operations?
- Yansura: Are you talking about a negative view?

- Hughes: No. I was thinking that when you are a private company, without public stockholders, you don't have to answer to outsiders to the degree that you do once your stock is publicly held. There are a lot of rules and regulations about being a public company.
- Yansura: Yes, that is true. Before we went public, we were indebted to the venture capitalists who gave us money. So it simply switched from the venture capitalist to the investor out there.
- Hughes: So that wasn't a big change.
- Yansura: It wasn't a big deal.
- Hughes: I thought perhaps that you might feel more pressure to get scientific results, to get a product to keep the stock price up.
- Yansura: No, I don't think many people felt that way, at least most of the scientists. I saw the scientists' view of what was going on, and everybody immediately thought, "Now I can retire if I want at the early age of thirty," or whatever. It was really an odd feeling. You had struggled all your life, and you didn't know for sure that you were going to come out with a decent job that you loved. And all of a sudden in your early thirties or whatever, you could actually think about retiring. It was a novel thought.

Gene Expression

An Intriguing Challenge

- Hughes: Here you are twenty years later, still here.
- Yansura: Yes, I am still here.
- Hughes: Why?
- Yansura: Well, the practical side is that you realize that you have to have a certain maturity to retire.
- Hughes: You mean more than age under your belt? Psychological maturity?
- Yansura: Psychological maturity. I never had had much money in my life, and I didn't have the knowledge to know how to invest and feel secure in that sense. It takes many years to do that, and to diversify, and so forth. At that point, I decided that I was going to learn more about personal finance. You don't want to retire and maybe not have anything out there.
- Hughes: Did people do that?

Yansura: I don't know of anybody that retired. Dave Goeddel is still working. Now, he doesn't have to work if he doesn't want to. The other side of this is that the scientists had their passions for science, and they wanted to pursue that; that was important to them also.

Hughes: Was that part important to you?

Yansura: Yes. At that time, and for most of my career at Genentech, my passion has been in expressing proteins. I still find that fascinating.

Hughes: Why?

Yansura: Because it's such a challenge. When Dave Goeddel called me to offer me the job, one of the things he said was, "We have to not only put the gene together, to construct it, but we also have to express it." That I thought was an incredible challenge. It wasn't clear at the time what it took to express proteins. Some people thought you just put the gene in there, and it turns it into a protein, and it's just automatically like a normal protein. But it didn't turn out to be that way. It's much more complicated.

Expressing Growth Hormone

Hughes: Did you suspect that it wouldn't be easy?

Yansura: When Dave Goeddel cloned human growth hormone and expressed it I was surprised that it expressed so easily.

Hughes: Was that in *E. coli*?

Yansura: That was in *E. coli*. He had put the human growth hormone gene behind the lac promoter, put it in *E. coli*, and turned on the promoter, and he got out the human growth hormone. In hindsight we realize how lucky he was. He didn't even realize the problems that he just accidentally overcame.

Hughes: What were some of them?

Yansura: For example, he had no idea about what was involved in the translation of proteins. There's ten, twenty years worth of work that shows what's involved, and his construct simply got through all the translation bottlenecks. It turns out that human growth hormone folds fairly easily. He made it in *E. coli* cytoplasm where none of the disulfides form. When he broke open the cells, the protein tends to fold easily enough, the disulfides reform, and he got native growth hormone out. Most proteins do not fold that easily in *E. coli*; it's very challenging.

Host Organisms

- Hughes: Hence the use of yeast and other organisms?
- Yansura: That brings up another point. There was a period very early on in the biotech industry where we went from the idea that we would express things in *E. coli* to we would need to look at alternative organisms. The hepatitis B vaccine project is what pointed to that. Now, looking back over twenty years, you still see *E. coli* is used for quite a few protein engineering products. There are only a few hosts commonly used. There are mammalian cells and *E. coli*, and those are still the main two.
- Hughes: People use *E. coli* whenever they can because it is easy to maintain?
- Yansura: It's easy to use. If you're going to manufacture some protein pharmaceutical it's much easier to make it in *E. coli*. It's cheaper to deal with.
- Hughes: Your bibliography indicates that you worked on several expression systems.
- Yansura: I have really only worked on bacterial expression--
- Hughes: I thought I saw something about vertebrate culture.
- Yansura: The one exception is some work on the hepatitis B vaccine. There's a patent involved.
- Hughes: It must be the patent I'm thinking of. [looking at the list of patents in Yansura's bibliography] Yes, "Polypeptides in Vertebrate Cell Culture."
- Yansura: Yes, that was the hepatitis B vaccine work. It was a failure in *E. coli*.
- Hughes: Because it wouldn't fold right?
- Yansura: There were two problems. One had to do with the fact that the hepatitis B protein that people were interested in making was a complex; it was a particle. It was a 22-nanometer particle. It consisted of a lipid bubble, so to speak, with the surface antigen embedded in there. And that's what was used as a vaccine at the time.
- Hughes: Maybe we should save hepatitis B for next time.

Increasing Insulin Yields, Benchmarks, and Eli Lilly & Company

- Yansura: The way things evolved at Genentech, once insulin was expressed, there was a fair amount of work to increase the yields.
- Hughes: Did you work on increasing yields?

Yansura: Yes, I worked on that also.

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Yansura: Swanson and Eli Lilly had signed a deal to eventually lead to the commercialization of human insulin by recombinant means. The first stuff we had produced was in very very small amounts. Stephen Hall estimated in his book that it was enough to give a partial dose to a small mouse, or something like that. Now we were talking about making kilograms of this stuff. We knew that we didn't have to make it here at Genentech; Eli Lilly did. They were the major players in bovine and porcine insulin, and they badly wanted the human market. So we knew it was their real desire to get a hold of our insulin. The deal was written with benchmarks. They weren't going to give us all the money unless we made these benchmarks, and they involved making some of the individual chains. Here's one of the letters from Genentech to Eli Lilly on one of these benchmarks.¹

Hughes: [skimming letter] Ed Smithwick is Genentech's contact at Eli Lilly?

Yansura: Apparently. Mike Ross directly contacted Eli Lilly. We badly needed to reach the benchmarks because there wasn't enough money at Genentech to keep going without pulling in this money from Lilly. Bob Swanson was very adamant about making sure that we met these benchmarks, so there would be this sort of crash near the end of a deadline, "We have to get this material to Eli Lilly!"

Hughes: What did that mean?

Yansura: The benchmarks were written very explicitly: Lilly wanted so many milligrams of A-chain and B-chain, or they wanted the expression yields at some specified level. Apparently we met all of these benchmarks. At the time Dave Goeddel was interested in cloning the interferons. I was really interested in working on the hepatitis B vaccine. So we did this insulin work on the backburner, to some extent, because it wasn't very exciting. I isolated some of the B-chain and worked on increasing expression into fairly massive quantities.

Hughes: Would Swanson crack the whip and say, "You people leave your pet projects and get back to meeting the insulin benchmarks!"

Yansura: Well, the other projects were important too. Bob wanted everything. He would say, "If you don't have more things on your plate than you can accomplish, then you're not trying hard enough." He wanted you to have a large enough list that you couldn't possibly get everything done, and yet he wanted you to try.

¹Michael J. Ross to Ed Smithwick, December 20, 1978. (Yansura donation to the Bancroft Library.)

Work Schedules and Scientific Styles

- Hughes: How did you feel about that pressure?
- Yansura: Everybody at the time was fairly numb, and we worked long hours.
- Hughes: How many hours a day?
- Yansura: I have a hard time remembering. But my wife, Patricia, reminds me that it was very long, that she spent most evenings during the week by herself, and that I would come in around nine or ten.
- Hughes: And probably not good for much, right?
- Yansura: Yes, not good for much. I would eat, sleep, and get going again the next morning. Five days plus part of Saturday were expected. Everybody else was working hard so you just fell in.
- Hughes: Bob Swanson, from what I hear, went home at a reasonable hour. Is that your memory?
- Yansura: Yes, that's true. Most of the time he would leave way before we did. That was just the norm.
- Hughes: And you didn't think much about that?
- Yansura: No.
- Hughes: Science required longer hours.
- Yansura: It really did. Things were much more tedious and slower then, and everything was new, and everybody was learning. You just had to be immersed in this technology and work at it.
- Hughes: Did the way that you and Dave Goeddel work change from how you had operated in Caruthers's lab?
- Yansura: I worked with Dave on the insulin project and on the human growth hormone project. It was pretty much how Dave had always been. He was the head guy in charge of the project, so I would do a certain part of it. We worked well together in our own corners of the lab.
- Hughes: So there wasn't constant interaction?
- Yansura: No, everybody worked on their own thing. When someone got a result, then everybody would look at it.
- Hughes: I haven't asked about Dennis Kleid.

Yansura: Dennis Kleid was the other scientist at Genentech at the beginning. Dennis was very smart. He had come out of Khorana's lab, also with Marv Caruthers, so he had been in on synthesizing the tRNA gene. Dennis was a different personality. He was in some ways a little goofy. That's really what you needed because you had Dave Goeddel, super intense, and Kleid was just the opposite. He was making jokes. I went from working with Dave to working with Dennis after a little while. The main reason was that Dennis had started the hepatitis B vaccine project, and I started working on that and was very interested in that project. So I ended up working for Dennis Kleid for a number of years. I like Dennis very much. He's a super nice guy. In the lab he was sort of a disaster, and he really didn't like the scene in the lab because he was very sloppy and impatient. He was trained to do it, but his heart was not in lab work, and so it was better he was in his office.

Hughes: Is that where he was most of the time?

Yansura: That's where he was most of the time. He had worked on the insulin project. He had done a fair amount of the work, not as much as Dave, but that's not saying much. After that, Dennis became more withdrawn from working in the lab. By the time we worked on the hepatitis B vaccine he was pretty much in his office most of the time, and I was doing the work. The foot-and-mouth vaccine, again I was pretty much doing the work with others; he was in his office. In contrast, Dave Goeddel was in the lab all the time. He was very intense, out cloning interferons, and so forth. And Dave was very good in the lab.

The NIH Guidelines

Hughes: One of the background features of this period was the controversy that was going on about recombinant DNA. One of the aspects of that controversy was the NIH guidelines. How much attention did you pay to them?

Yansura: The NIH guidelines maybe didn't have as strong an effect on us as they had on the academic labs.

Hughes: Why was that?

Yansura: For example, Wally Gilbert and Howard Goodman were our competitors in the insulin race. To clone the human genes they had to use a P4 facility, which is very difficult to even find. Our stuff was a synthetic gene; a synthetic gene for a human protein. It wasn't the actual human gene; we used different codons and didn't have the pre or pro parts of the actual human gene. In some ways it was absurd, but it was considered non-human. So we were able to clone this in the open and not have all these restrictions. That was ultimately to our advantage. The restrictions eventually got lifted so you could clone human DNA in any way after a while. For the most part they weren't a problem. When we worked on the foot-and-mouth vaccine there were guidelines and we had to work in a P4 facility.

- Hughes: There was also a ten-liter batch limit into the 1980's which I would think applied to the scale-up procedure. Do you remember that?
- Yansura: For us, ten liters is a lot of stuff. For anything we did in the lab, ten liters was a humongous volume. Even nowadays, we consider that large-scale for any application. It's not until you're getting into manufacturing that you need to go up to 100 or 1000 liters or more.
- Hughes: So the volume limit didn't affect you in the lab?
- Yansura: No, not at all.
- Hughes: Insulin was being developed and scaled up by Lilly. So they were the ones that had to worry about the ten-liter limitation?
- Yansura: Yes, that's correct.
- Hughes: By growth hormone that limit had disappeared, I believe.
- Yansura: I believe that's true.
- Hughes: So the NIH guidelines didn't have much impact on Genentech.
- Yansura: Yes, that's correct.

Recombinant Hepatitis B Vaccine

[Interview 2: October 10, 2001]##

Merck's Blood-derived Vaccine

- Hughes: Today we agreed to start with a discussion of hepatitis B. Why did Genentech decide to get into that project?
- Yansura: I think it was obvious that making a recombinant vaccine for hepatitis was a logical step. At the time, everybody was looking at proteins that were either pharmaceuticals or would be close to becoming pharmaceuticals. These were the easy pickings in terms of proteins that could be turned into pharmaceutical products.

At the time, the hepatitis vaccine was made from a component of human blood. They would use the blood of carriers of hepatitis, and they would extract out a protein or an antigen for the hepatitis virus. This antigen was a small particle like a lipid, a little particle bubble, so to speak, with the hepatitis surface antigen on the outside. It was

essentially one protein, but it was a protein that had a span of amino acids that went into the membrane and anchored it in there.

Hughes: Was all that known as you began the project?

Yansura: Yes, this was all known. I believe it was Merck which was selling the vaccine that was made from the serum of carriers.

Hughes: Yes it was. [Maurice] Hilleman was at Merck and had a role in making a blood-derived vaccine.

Yansura: Apparently it worked very effectively. It was a fairly good vaccine, but it always carried the risk that you would get hepatitis or some other disease from it. Later on, everybody was worried about getting AIDS from this vaccine. Anytime you get a product from blood you always worry about something going wrong.

Hughes: Aren't all children and health care workers in this country now immunized with the recombinant vaccine?

Yansura: Yes, that's true. It's much more widely used now. At the time, it was primarily used by medical care personnel. That was obvious because the virus is transferred through blood and body fluids.

Hughes: Was the reason for the circumscribed use of the older vaccine because there was a risk?

Yansura: I'm not sure that was the only reason, but I would think that would be the major case. I would be hesitant to take it knowing that it was made from carriers of hepatitis. I'm not sure that was in the equation when they decided who should get the vaccine and who shouldn't. But I'm sure that was in the back of people's minds.

Hughes: Had the surface antigen been cloned?

Yansura: No. At the time the old vaccine was being used, recombinant DNA technologies were just starting to come on board. As soon as they were on board, everybody looked at insulin first. After insulin was on its way, people were looking for other obvious candidates, and certainly hepatitis was one.

The Genentech-Merieux Recombinant Vaccine

Hughes: Do you remember when Genentech first began to work on hepatitis?

Yansura: I believe it was 1979. I might be off by a year or so. We got into a deal with another pharmaceutical company to make it recombinantly, and that's how we got started.

Hughes: Do you remember the name of the company?

- Yansura: I believe it was Merieux, a French company. Dennis Kleid dealt directly with Merieux, and he wrote all of these reports that I have here.
- Hughes: What was Genentech expected to do, and what was Merieux going to do?
- Yansura: They wanted to produce a recombinant vaccine for hepatitis B. Beyond that there wasn't a lot of close communications as to exactly what they wanted, but I think in general they just wanted a vaccine that was safe, made by recombinant means, and a way to produce a lot of the surface antigen.
- Hughes: Do you know anything about the contract negotiations?
- Yansura: I don't know very much about the contract. I think when you talk to Dennis Kleid he'll have a lot more information.
- Hughes: How did you come to work on this project?
- Yansura: The insulin project was moving along--we were trying to improve the yields on it and so forth--so there was a need to bring in new molecules. [animal clock chimes]. There were several projects that were started almost immediately after the insulin was announced. One of these was human growth hormone, and the second was probably the vaccine for hepatitis B.

The Process for Initiating Research Projects at Genentech

- Hughes: How did these projects come to be? Was it the scientists saying to Swanson, "This is an obvious protein, and here's a disease that needs a vaccine." Or was he out there fishing around for new areas to explore?
- Yansura: That's a good question. I'm sure new projects were started by both Swanson and by individual scientists. Some of the projects picked early on, such as human growth hormone and insulin, were obvious since they already had a market. I'm not sure how hepatitis came to Genentech; if Merieux came to us or if we came to them.
- Hughes: The project could have come from the industry side?
- Yansura: It could have come from the industry side.
- Hughes: But when projects were originated internally, such as the insulin or the growth hormone, would it be the scientists that started the idea rolling?
- Yansura: I think nowadays that's true because nothing is obvious anymore, and so you need a scientist to find something that's maybe interesting and really be a pusher of this idea. In those days there were these so-called low-hanging fruit projects that were just obvious, like insulin, growth hormone. These were all proteins that were already used as

pharmaceuticals. So it was obvious to say, "Okay, if we make this by recombinant means we'll have a great advantage over the old methods."

Hughes: Would you include the interferons in that easy-picking range?

Yansura: Interferons were slightly more removed in that they were interesting proteins at the time, but they weren't used as pharmaceuticals. You had that additional uncertainty of whether they would actually be a pharmaceutical.

Hughes: Is that one reason why Genentech's research on the interferons came a little later? Maybe I'm wrong about that; Dave Goeddel was always interested in interferon, wasn't he?

Yansura: Yes.

Hughes: So as soon as he could get away from insulin he wanted to work on interferon?

Yansura: Or human growth hormone.

Hughes: Oh, human growth hormone was it?

Yansura: Yes. The interferons were known proteins at the time, but there wasn't a lot of data on the sequence for the proteins, and they weren't used as pharmaceuticals. So there was still that uncertainty whether they could become a pharmaceutical product.

Hughes: One of the problems was that the interferons were only available before recombinant DNA in minuscule amounts, right?

Yansura: Yes, that's true.

Hughes: So even to do the science was difficult, let alone to figure out their therapeutic value?

Yansura: Yes. For the interferons, I think the therapeutic use was always thought to be in terms of some kind of cancer therapy. There wasn't much protein around, and the sequence wasn't known. For many of the other proteins, insulin for example, the actual amino acid sequence was known before the project even started.

Hughes: Getting back to your involvement with hepatitis B, how did it work in those early days? Were scientists assigned to projects, or was it a natural evolution: You were finishing up on insulin, and you needed another project, and why not hepatitis B? Or was it more systematic than that?

Yansura: It was more of the first route you suggested. It wasn't very organized; it was simply everybody picking up the next thing, no matter what it was.

Problems in Protein Expression

- Hughes: Did you have a particular interest in working on hepatitis B?
- Yansura: I didn't have any real great interest in hepatitis B at the time. I was becoming interested in just expressing proteins. When Dave Goeddel had expressed human growth hormone directly, as we called it then, without any leader sequence or fusion partner, that was quite a shock to see that that could happen. I wanted to do something like that also. The hepatitis came along, and I viewed it as another protein.
- Hughes: It wasn't quite that easy, was it?
- Yansura: It was not that easy. It turned out that the expression was probably the most difficult part of the project and of turning it into a pharmaceutical.
- Hughes: What were the roadblocks and how did you get over them?
- Yansura: There were several groups working on a recombinant hepatitis B vaccine, and the sequence for the virus was well published. It's a DNA virus, and I think there were three to five genes, so it was very small. Everybody wanted the hepatitis surface antigen. This is the protein that sort of sticks out of the virus. In the vaccine that Merck was making at the time, it was the protein that stuck out of these small 22-nanometer particles. This is what drives your immune response.
- Hughes: That was all known?
- Yansura: Yes, that was all known. So all the basics were there, and the sequences of the whole genome came out fairly quickly. I was surprised that sequence data would come out that quickly, but it did come out. Then the next obvious step was to take that sequence, somehow make a construct for expression, express that protein, and that protein could essentially be a vaccine. But it wasn't that simple. To this day, it's still a problem in that the protein has a span of amino acids called the transmembrane sequence. These sequences are particularly difficult to express.
- Hughes: Why?
- Yansura: I don't know. [laughter] In fact, we are dealing with a number of similar proteins now as tumor antigens, and they have the same type of transmembrane sequence. Normally they're made in the cell; they're inserted right into the membrane as soon as they're made. For some reason, that's a very difficult step to do. The other way we can express proteins in *E. coli* or any other organism is not put them in the membrane, just put them in the cytoplasm. That's very difficult also for some reason.
- Hughes: The biological basis for the difficulty is not known?
- Yansura: That's correct. It's somewhat surprising that twenty years later people are still struggling with these proteins. One of the reasons is that it wasn't that critical for most proteins to

make the actual transmembrane section as long as they made the big extracellular domain. Those are easy to express in almost any organism. But once you start attaching the transmembrane section it becomes much more of a challenge.

Hughes: You have to have that transmembrane section for the protein to function correctly?

Yansura: No. At the time, everybody was trying to make something that was very close to these 22-nanometer particles that Merck was using for their vaccine. If you try to make something like that, you need the lipid bubbles, so to speak, with the surface antigens sticking out. To keep the surface antigen tethered to that lipid bubble you need the transmembrane [section]. In principle, you should be able to cleave it off and just use the extracellular domain.

Hughes: In principle, but not in fact?

Yansura: I don't know if anybody has done that experiment with hepatitis. With other proteins you don't really need the full transmembrane part. So the way the project was working from everybody's angle is, nobody really knew quite what to do. Everybody was trying to ultimately make what Merck was selling, which was this lipid particle with these hepatitis surface antigens sticking out.

Protein Expression

Development of Different Expression Systems

Yansura: To try to make this protein, all these different expression systems evolved very quickly. Throughout the insulin project, nobody talked about anything but *E. coli* in terms of expressing the protein. When the hepatitis vaccine project came into focus, and as the project started moving along, it was clear that you couldn't make everything in *E. coli* that easily and that we were going to need multiple expression systems. It was the hepatitis project which started the yeast expression systems, the mammalian expression systems, and of course the *E. coli* systems were still involved in that also.

Hughes: Was it?

Yansura: Yes.

Hughes: You mean for things other than hepatitis?

Yansura: Even for the surface antigen. We eventually expressed it at Genentech once we removed the transmembrane part. I believe it was Pierre Tiollais' lab at the Institut Pasteur which expressed the whole protein with the transmembrane as a beta-galactosidase fusion. He was able to make some of the complete protein.

Protein Folding

Yansura: The second problem was protein folding. That was a big unknown at the time: How to deal with recombinant proteins if we also have to fold them?

Hughes: Folding hadn't been a problem with insulin?

Yansura: Insulin was folded after it was purified, so it was folded in vitro.

Hughes: Spontaneously?

Yansura: It's much more difficult than that. People had been playing with insulin for years. It was discovered in the twenties, or something like that. There was a lot of insulin around for protein chemists to get a hold of. They could reduce it, separate the chains, recombine it, and get active insulin out. There was a lot of work that had gone into being able to play with that protein.

With growth hormone, we lucked out in that it folded fairly easily. It's one of these proteins that folds fairly quickly in *E. coli*. The hepatitis surface antigen, on the other hand, was much more difficult. We didn't have a very extensive protein chemistry group at the time which could refold proteins, and so we didn't know what to do with it as a misfolded protein.

Hughes: And misfolded is equivalent to nonfunctional?

Yansura: Well, that's not exactly true. The foot-and-mouth vaccine protein was not folded, and it was injected into cattle and pigs, and it gave a great immune response. It's very tricky; some proteins you can get away without folding, and some you have to for vaccines.

Hughes: To have a protein fold in the way you wish is equated with finding the correct expression system?

Yansura: Nowadays we can refold just about any protein in vitro, so we don't really care about which system is used anymore. Back in those days, ultimately it wasn't clear if we needed the hepatitis surface antigen to be folded or not, although everybody was going towards the 22-nanometer particle. That was the gold standard because people knew that that worked as a vaccine. So if you could remake that, you would have a vaccine. If the surface antigen was misfolded, you might still have a vaccine but you didn't know that.

Hughes: Was it the 22-nanometer particle that was immunogenic in the older non-recombinant vaccine?

Yansura: Yes.

Hughes: So it was obvious when you were trying to build the recombinant vaccine to think that the surface antigen was where you should be headed.

Yansura: Yes, because the 22-nanometer particle vaccine had gone through the FDA, and it was an approved vaccine. Everybody was trying to make that because once they made that, they knew they had a vaccine.

Hughes: They were home free.

Yansura: They were home free.

Acquiring Expertise in Yeast and Mammalian Expression Systems

Hughes: You at Genentech must have had to adopt a new expression system for the first time.

Yansura: That's correct.

Hughes: How did you learn how to do that? The UC San Francisco group, to acquire that technology, had formed an alliance with Ben Hall at the University of Washington.

Yansura: That's correct.

Hughes: So what did Genentech do?

Yansura: We hired a yeast person. His name was Ron Hitzeman.

Hughes: Had he come from the University of Washington?

Yansura: I can't recall where he came from, but he definitely came out of a yeast lab.

Hughes: And were there plenty of those around?

Yansura: There was a fair number of them. Maybe there were only a few that could try to make recombinant proteins in yeast. Making recombinant proteins in *E. coli* had just started, so there was maybe a year or two of experience there. All the other systems were a little farther behind. Yeast, as a eukaryote, is more complicated. But it was clear that during the hepatitis project all these other expression systems came out of the woodwork so to speak.

Hughes: Yeast is a different culture system, and Genentech didn't have very much space in those early days.

Yansura: You mean lab space? Well, yeast sort of looks like bacteria; you grow it in shakers.

Hughes: So you can use the same sort of equipment?

Yansura: Yes. It's nothing fancy.

- Hughes: So what happened? You had a lab that became a yeast lab?
- Yansura: We were the focus of *E. coli* expression. We hired Ron Hitzeman to do expression in yeast, and we hired Art Levinson to do expression in mammalian cells.
- Hughes: There weren't many people doing expression in mammalian systems, were there? What is this, 1980?
- Yansura: It must have been either 1979 or 1980. Genentech was expanding at that time.
- Hughes: In 1980 Genentech went public.
- Yansura: I believe Art Levinson and Ron Hitzeman came before it went public, but I'm not positive. Their expertise was to create alternative expression systems. We didn't know at that time what was going to be required to make all the proteins we wanted to make. Even to this day those three expression systems are the main expression systems. The yeast has fallen somewhat over the years, due to some problems.
- Hughes: Tell me about mammalian expression systems and how widespread that know-how was in 1980.
- Yansura: There was a fair amount of knowledge in working with mammalian cells in culture. That wasn't a problem. There was a number of labs out there, particularly in the medical sciences, that used cell culture. Now, it's one thing to grow these cells in culture and another thing to get genes inside them, and expressed as proteins.
- Hughes: So talk about that, please.
- Yansura: At the time, expression technology was fairly crude. You took the gene of interest and you fused it to a promoter. Usually the promoter had been studied a little beforehand. So some basic bits of knowledge were known about it. You fused these two pieces of DNA together; you stuck them in the organism somehow; you crossed your fingers.
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- Hughes: What mammalian cells were you using?
- Yansura: I didn't do any of this myself. But there were similar methods to get DNA into these organisms. I can't recall exactly what they did; I believe it was some kind calcium-phosphate treatment for the mammalian cells.

Genentech's Empirical Approach to Expression

- Hughes: Was expression a problem?

Yansura: Expression has always been a problem. [laughter] In those early days we just didn't know; we just took a promoter, fused a gene to it, and threw it in there. It is after years and years and years of playing with this that we finally understand a little bit more about this. A lot of basic knowledge is now available for *E. coli*. As you get to the higher organisms, like mammalian cells, even to this day there isn't a great amount of knowledge available in terms of what's going on or what the problems are when expression doesn't happen. It's still sort of a black-box approach.

Hughes: Is that lack of knowledge because it's so complicated, or is it because people are less interested in that as a problem to unravel?

Yansura: I think people are interested in unraveling the problem, but it turns out that it's much easier to work with *E. coli*; you can figure out what the problems are--if it's at the translation level or the secretion level or the folding level etc. All that can be sorted out now, and you can do those experiments usually in a couple of days. If you have the plasmid already made you can get protein expression in maybe four or five days. In mammalian cells you can't do that because it takes so long to do the experiment; it can take months. The way they insert the gene or the DNA plasmids into mammalian cells, they don't always have very good control of how many copies get in there, and they don't know where they go. There's a lot of uncertainty, so it's hard to do experiments where you manipulate one thing and see what effect it has on expression; whereas in *E. coli* we can do that fairly easily. I think in yeast, you can easily do a fair amount of work there as well.

Hughes: Could you say something about how the technology developed as you go from project to project?

Yansura: I think I've already made it clear that the hepatitis project really presented all the protein expression problems very early on in the industry, in other words, to produce proteins in folded forms, and so forth. This resulted in the radiation of all these different expression systems, primarily *E. coli*, yeast, and mammalian cells. Later came *Bacillus* and insect cells.

I think you asked, was there something that happened which influenced our thinking about the expression systems? Yes, that did happen. In the hepatitis case, everybody thought that we would make every protein in yeast for a while. This happens a lot in the industries: People get these preconceived notions that this is the only way; this is the only door to the future. The reality is that it's usually false. There are a lot of different doors, and there is no one door to make all these proteins.

Hughes: How soon do you think you came to that realization?

Yansura: It took me twenty years. [laughter]

Hughes: Was the assumption that yeast was going to work better because it's a eukaryote and you were trying to produce human proteins?

Yansura: Yes. There was sort of a religious tone to it. The thinking was that here you are, trying to make a human protein; you want to get as close as you can to a human to make that human protein. It's sort of this warm fuzzy feeling about this protein, which is totally nonsense and totally nonscientific; there's no basis to this at all. But there is a feeling among a lot of scientists that to make a human protein you need all this massaging and care that you can only get from a eukaryote, and the closer you get to a human cell the better it is. To some extent that's true, but to some extent that's total nonsense. There was no scientific basis for it; it's just this feeling that people got.

At the time of the hepatitis work, yeast looked like it could produce this 22-nanometer particle or something very similar to it. You would hear people say that yeast expression was going to be the way of the future; this is the way all proteins are going to be made. Obviously that was false. It wasn't based on any scientific knowledge; it was just this feeling that people had.

Hughes: This was a general feeling in molecular biology?

Yansura: I'm talking from my viewpoint at Genentech because obviously that's where I was, and we had a fair amount of scientific personnel at the time. You assume that other people outside Genentech were seeing the same thing, but maybe that's not true.

More on Hepatitis

Not a Prime Genentech Project

Hughes: Who was working on the hepatitis project aside from you?

Yansura: In the early days it was primarily me. If you look at these reports that Dennis Kleid wrote, he would try to put as many names as possible on the list of people that were involved. These reports were going to Merieux, and he wanted to make it look like we were putting a lot of effort into that. But if you read what was in the reports, basically it was all my lab work.

Hughes: Well, I was deceived. I thought, that's pretty good for a young company to have so many people on this project. [laughs]

How much were you relying on consultation with people both inside and outside Genentech? In other words, how isolated were you?

Yansura: We did have outside consultants, but for the most part the answers just weren't there. This was a new area, and it was anybody's guess.

Hughes: Were you talking about your problems within Genentech? With Dave Goeddel, for instance?

- Yansura: I think everybody at the time was bogged down in their own problems. We did talk to each other, and we did communicate in the general sense. But Dave Goeddel was doing interferons, and he was competing against all these other groups; he was under the gun. He was pretty much thinking of his own problems. And I think that was true of everybody.
- Hughes: Were you feeling the competition?
- Yansura: Yes, obviously. We had competition from William [J.] Rutter's group, and they essentially came out pretty much on top of this project, although we did get some patents on expression in yeast and mammalian cells. So yes, there was lots of competition. But at Genentech the hepatitis project wasn't considered something we really had to get.
- Hughes: Why was that?
- Yansura: I think the main reason was that it was a contract with another company. We had growth hormone which we had decided we were going to keep for ourselves, and those are the projects that really bring in the good money. Anytime you do a contract with another company, those projects never have the backing as does an in-house project.
- Hughes: And yet growth hormone was to receive orphan drug status. The potential market was rather limited, wasn't it? Particularly compared to hepatitis B vaccine.
- Yansura: Yes, that's true. At the time we estimated that growth hormone would give us in the tens of millions of dollars, which is nothing really for a pharmaceutical company. The inside joke at that time was that it would pay for the toilet paper. The hepatitis B vaccine now is fairly lucrative. It makes a lot of money.
- Hughes: It's the largest income patent at UCSF. I have no idea how it measures up to lucrative patents in general.
- Yansura: Yes. Ultimately both of those projects made a lot more money than anybody at first thought. The hepatitis vaccine, at the time, was only being given to health care workers, so it wasn't a huge market either. Nowadays, of course, grade-school and high-school kids get it. In some ways UCSF and Merck both lucked out. We also lucked out with growth hormone in that we eventually were able to pretty much get a much larger market than we initially thought.
- Hughes: You, Dan Yansura at Genentech, were carrying the hepatitis project. At what stage did it go over to Merieux?
- Yansura: I was doing most of the early *E. coli* expression work, and we could not make much protein in *E. coli*; it was misfolded, we didn't know how to deal with it. Ron Hitzeman and Art Levinson both brought to Genentech their expression systems, and we moved the gene from *E. coli* into these alternative expression systems. Both of those systems expressed the hepatitis surface antigen as a 22-nanometer particle.

Genentech's Vaccine Division

Liability and Competition

- Hughes: Fairly early on, I think by 1983, Genentech was working on an AIDS vaccine. What was the overlap, if any, between these two vaccine projects in terms of personnel and approach?
- Yansura: We had a whole department that was dedicated to vaccines. It was somewhat controversial in that Bob Swanson was not totally on board that we should have a vaccine department, and the reason is that vaccines do not make much money.
- Hughes: They also pose a liability problem.
- Yansura: Yes. So any money they do make, they lose quite a bit to liabilities.
- Hughes: Did Chiron enter into Swanson's thinking? The fact that a near neighbor had set out to focus on recombinant vaccines.
- Yansura: Chiron was initially based on Rutter's work with hepatitis B. They used that success to build a company.
- Hughes: Vaccines were a deliberate target at Chiron, which was not a common way for the early biotech companies to go. You're saying that at Genentech vaccines were not a heavy interest?
- Yansura: Yes, they were not big moneymakers. On the other hand, they were the easy pickings in which to use this new recombinant technology. So the scientists tended to want to go after the vaccines because they were obvious candidates; they were right there. In almost any disease caused by a virus, everybody takes the surface protein and uses it as a vaccine. It doesn't take much brains. The same thing happened with the AIDS virus. When the DNA sequence became known after the virus was identified, it was obvious everybody would go for the protein on the outside of the virus particle and try to make a vaccine out of it.
- We had a whole vaccine department dedicated to making vaccines. It started with the hepatitis B project. Eventually foot-and-mouth became a major project in that department, and then ultimately the AIDS vaccine.
- Hughes: Did that mean hiring a new set of scientists, or were people transferred from other subdivisions of the company?
- Yansura: We were growing at a fairly high rate then, so we were always adding new people for new projects.

Collaborating to Acquire Expertise

- Hughes: Was it easy to find people with expertise in vaccinology?
- Yansura: Nobody really had expertise in recombinant vaccines. That was true of the whole industry at the time. When we had the insulin project going, we weren't experts on insulin. We knew very little about insulin, except we knew it was a protein diabetics used, and we might be able to make it by recombinant means. We didn't know much about growth hormone either. Or the interferons. People had studied the interferons for years and years; we came along and we just wanted to clone it and go from there.
- Hughes: How do you think scientists who had been working in these fields for maybe decades felt about you upstarts coming in and not treasuring the basic science but just going for the goods, so to speak?
- Yansura: Yes, going for the gold. I think they hated us. You could see why.
- Hughes: Did you feel any of that yourself?
- Yansura: What do you mean?
- Hughes: People who had been working for a long time in the hepatitis field might have expressed, at meetings or whatever, some resentment towards the Genentech project, and hence towards the people working on that project because they were new boys on the block.
- Yansura: In most of the projects, in fact all the projects, we always collaborated with some group that was an expert in that area. With the insulin project, we collaborated and had a deal with Eli Lilly. They were the major producers of insulin at the time. So we were working together there. With the hepatitis, we were working with Merieux. With the foot-and-mouth vaccine, we had a collaboration with the U.S. Department of Agriculture in New York. We would always work with groups that were experts in that area. Our expertise was with recombinant DNA.
- Hughes: That's interesting what you just said, but I was picturing the basic scientists who had been struggling along in their academic labs for years, trying to work out the natural history of hepatitis B virus or whatever. And then all of a sudden there were these young scientists who didn't particularly care about all this background work; you just wanted to clone and express the surface antigen and make a vaccine.
- Yansura: Yes, and make money.
- Hughes: And make money.
- Yansura: That's true; that was our approach. But on the academic side, most of the labs realized what they were facing. They brought recombinant DNA into their labs and incorporated it. Now pretty much all these labs in medical science have recombinant expertise. So the competition really spread the technology around.

Dissemination of Recombinant DNA Technology

- Hughes: You and others at the front edge of the genetic technologies pushed the scientists who had been in the field prior to recombinant DNA towards adopting these new technologies because to survive they had to?
- Yansura: Yes, that's exactly what happened. If they were going to survive, they had to bring in recombinant technology into their labs; they had to be able to clone the genes that they were working on, and so forth.
- Hughes: Were Genentech scientists going to meetings and talking about what they were doing?
- Yansura: Yes, there was some of that. The academic side wasn't as pronounced as it was if you were in academia, of course. Most of the work was put out there primarily through publications. We had a press conference to announce our insulin success, and we took a lot of flak for that.
- Hughes: Were you there?
- Yansura: Yes, I was there.
- Yansura: I think tomorrow's the last day of the City of Hope v. Genentech trial.
- Hughes: Let's hold that discussion. [See interview number four.]

Yet More on Hepatitis B

Merieux, Litigation, and Clinical Trials

- Hughes: In what form did Merieux receive the prototype vaccine material?
- Yansura: We had produced these particles in both yeast and mammalian cells, and they were presented with the protein in that form. That apparently was what they were interested in. They wanted something that was very close to the vaccine that was out there at the time; they looked very close to that 22 -nanometer particle. We eventually produced that for Merieux. I am not sure where they went with that. Apparently Chiron has the market.
- Hughes: There is also a hepatitis B vaccine made by SmithKline. It's not yours?
- Yansura: I don't know where theirs comes from. We had patented the expression of the surface antigen in both yeast and mammalian cells. Very soon after that we got into a legal battle with Chiron on a patent interference proceeding. Dennis Kleid will tell you more about

that because he eventually moved to the legal department at Genentech and has been working on that case for almost twenty years.

Hughes: It's still pending?

Yansura: The last time I talked to Dennis it was still pending.

Hughes: What does it hinge on?

Yansura: We use very similar technology. The interference/patent situation involves making the hepatitis surface antigen in yeast. They made it in yeast, we made it in yeast. It came down to the point of whose notebook had the earlier date, suggesting that they had done it first. These things are difficult because you are never quite sure what exactly that actual date is. So the case has gone on for years and years. There were a number of us who gave depositions for this case. It just kept going on and on. Most of us forgot about it because we'd just move on. You can't just think about something for twenty years.

Hughes: A stumbling block to research on hepatitis B was the fact that there wasn't an animal model. Did that make any difference to you?

Yansura: No. And the reason is because we weren't really prepared to do that kind of work.

Hughes: That would be the clinical trials?

Yansura: Yes, that would be preclinical and clinical trials. When we got that far, Merieux would do those kinds of studies because they were situated to do that kind of work.

Hughes: Is that true to this day? No, it's not true to this day. You sponsor clinical trials.

Yansura: Yes, and we have animal facilities; we use mice, monkeys, chimps, and so forth.

Hughes: When did all that start?

Yansura: It started fairly soon, certainly by the time we started bringing growth hormone on board. Then for all these later projects we obviously brought in animal facilities, at least small ones, to deal with projects.

New Methods in Vaccinology ##

Yansura: Synthetic DNA continuously plays a major role in everybody's projects these days, but over the years it's been taken for granted. It's incredibly important for everything we do today, and it's hard to overestimate that. On the other hand, it's so routine now: to order synthetic DNA, we go on the computer; we fill out the form; and then four days later we get tubes sitting there waiting for us. So we don't think about all the work and the technology that went into DNA synthesis.

In terms of the hepatitis B project, beyond the expansion of the expression groups--there was the *E. coli* system for the insulin/growth hormone work now expanding to yeast and mammalian cells--some new methods to make vaccines had come out. One was Richard Lerner's work at Scripps. He had come up with the idea that you could make vaccines by simply synthesizing small peptides of the antigen. In other words, you don't need the whole protein since only small parts of that protein actually are the epitopes for making the antibodies. He tried this technique for a number of vaccines. He would make small peptides, try them out, and see if they could be used as a vaccine. This was an interesting idea, and there was initially a lot of excitement around it, but eventually it faded. I haven't heard anything about it for ten years or so.

Hughes: When was this approximately?

Yansura: 1981 is when he had first produced some peptides from the surface antigen and showed that you could inject these and make antibodies against the native hepatitis surface antigen.

Hughes: Did you try that method?

Yansura: No, we didn't. It was a new technology that was shooting off in a different direction. Our expertise at Genentech has always been in making recombinant proteins--full large proteins--and this was a new technology to make just part of these proteins. I think most of us felt that this was Lerner's stuff, and if it was going to be successful, he had all the patents on it already. It wasn't worth it for us to go that route.

The second idea that came out for making vaccines was from Bernard Moss's group at the NIH. He came up with the idea of using a live vaccinia virus as a base in which you express alternative proteins, for example, the hepatitis surface antigen.

Hughes: Why vaccinia? [Animal clock chimes] I'm glad I'm not interviewing you at noon. [laughter]

Yansura: Vaccinia was used for many years as a vaccine for smallpox. So there was a lot of knowledge about it, and people knew how to use it. Bernie, as we would call him in our lab, had come out with this idea of including additional viral surface protein genes integrated in the vaccinia DNA. In other words, let's put the hepatitis B surface antigen gene in there and express it at the same time. I believe he tried several other vaccine ideas with this vaccinia virus. That was a new technology, an offshoot of recombinant DNA. It was very exciting at the time, and it looked like that would perhaps be the way to produce all these vaccines. Well, it has slipped over the years. I haven't heard anything for quite a while so I assume that it died out.

Hughes: Genentech didn't adopt that approach?

Yansura: If this was an approach that we had really wanted, we would have had to license it. You can do that, especially if it's not another company. Bernie Moss was at NIH, and so you could probably get some kind of patent license to use his technology for certain vaccines.

- Hughes: But you'd want to be pretty sure before you spent the money on the license that it was going to be a better approach?
- Yansura: We use a lot of licenses now that don't pan out. A lot of the licenses have clauses in them so that you only have to give the licensor a small amount of money unless it really works.
- Hughes: Were you, because you were the main person on this project, watching for new methodologies with the idea that maybe they should be licensed?
- Yansura: Dennis Kleid was doing all of the coordination of the hepatitis project. He dealt with Merieux; he wrote up the progress reports for Merieux.
- Hughes: He watched the literature, whatever methods there were, to find out what these other groups were doing, like Moss's?
- Yansura: Yes. Most of these ideas came out in *Nature* or some other journals, so you couldn't miss it. By this time we had quite a vaccine group going and had moved beyond the one project.
- Hughes: Were you trying to keep up with the literature, or was that Dennis' responsibility?
- Yansura: It's impossible to keep up with the literature. [laughter]
- Hughes: So how do you handle the situation?
- Yansura: You just do the best you can. I don't know anybody that really keeps up with everything. There's just too much out there, and if you do try to keep up, then you don't have the time to do anything else.
- Hughes: How do you decide when you have done enough reading to do what you need to do?
- Yansura: Well, let me put it this way, you have to produce enough of your own work to stay in business, and if someone else comes out with a great idea, it's nice for you to figure out what he's done and understand it. But it's his idea; and no matter how well you understand someone else's idea, you're not going to get the credit for it, and you can't use it easily in a situation like this. So you don't want to spend too much time following what everybody else is doing. You want to know what they're doing in a very quick way, but you want to be focused on your work, create your own inventions, so to speak.
- Hughes: Is that approach more pronounced in industry?
- Yansura: In academic labs you also have to know what everyone else is doing, but you have to produce your own original work as well, so you have to put enough time into doing that. You can't really survive if all you do is know what everybody else has done.

Hepatitis B, the "Silent" Epidemic

- Hughes: I read that hepatitis B was considered in the seventies and most of the eighties to be a silent epidemic; people, for one reason or another, didn't talk much about it. Were you aware of this?
- Yansura: There was obviously a number of people infected. Merck would screen blood and find carriers and make their vaccine, so there were enough of those people out there. I think the reason it was silent is that most people recovered. They got sick for a while, they recovered, and that was it. A small percent of those ended up developing complications, such as liver cancer.
- Hughes: The three companies that came out with recombinant hepatitis B vaccine were, in order, Genentech, Biogen, and Chiron. Was Genentech's foot-and-mouth vaccine approved before hepatitis B?
- Yansura: No. We never had our own vaccine at Genentech. We never had a successful vaccine.
- Hughes: You don't consider foot-and-mouth to be yours?
- Yansura: It was ours, but ultimately it failed. All of our vaccines in some ways have failed. Even our AIDS vaccine was spun off because it didn't look promising. We just didn't have much luck with vaccines.
- Hughes: But that's not a problem unique to Genentech, is it?
- Yansura: No. I'm not aware of many recombinant vaccines other than hepatitis B. There were efforts to make recombinant influenza vaccine; you don't see that out there. The AIDS vaccine is still in limbo.
- Hughes: What about pertussis, that trivalent vaccine for children. Is that recombinant?
- Yansura: I'm not sure of that. Maybe there are more out there, but I'm not aware of them.

It seemed early on that recombinant vaccines were not going to be as easy as people originally thought; they weren't a sure thing. So we got out of the vaccine business fairly quickly.

Foot-and-Mouth Disease Vaccine

Project Origin

- Hughes: Tell me how Genentech got into the foot-and-mouth disease vaccine project.

Yansura: Dennis Kleid became very interested in vaccines early on. He worked to get and coordinated the Merieux contract. Right after that he became interested in expanding this idea: Can we make other vaccines? This was true of a number of other companies and labs. Everybody was thinking the same thing: you could use recombinant DNA to make a protein. The idea was that you would make the protein that was on the outside of the virus and that could be used as a vaccine. It wasn't any great thinking; it was so obvious then. Everybody looked at all the different viral diseases out there and thought about which ones could be [susceptible to] this new technology. Foot-and-mouth was on that list.

Hughes: Why?

Yansura: One of the reasons is that the existing vaccine was made from live virus. I believe it was heat killed. You took a live virus and supposedly killed it and used that as a vaccine. Some of the virus apparently did not always die. Most people in the field thought that a number of outbreaks occurred because the vaccine wasn't perfectly killed. So that was a perfect case where we could make a recombinant protein that was used instead of the whole virus; there was no way you could get outbreaks from this kind of vaccine.

At the time, foot-and-mouth was not a problem in the U.S. In fact, it has not been a problem in the U.S. for many years. I think there was one outbreak quite a long time ago. There wasn't any market for the vaccine in the U.S., but in Europe and in South America there was a fairly huge market.

Dennis Kleid became interested in not only this one but other vaccines as well. He had a bunch of ideas going, including influenza. But foot-and-mouth somehow took off. He had talked to some people at the USDA [United States Department of Agriculture] on Plum Island in New York who work on foot-and-mouth. This is the only place in the U.S. where anyone is allowed to have the virus. These researchers were very excited about working with us to make a vaccine. They had no technology at all to do this. They were strictly using the old biological process; they could grow virus and analyze some of the proteins and so forth, but they had no ability to do recombinant DNA work. So it was a perfect match. They were very interested in it, and for us it was an opportunity to work with a group that knew what they were talking about. We really didn't know much about foot-and-mouth. And this was true of all of the products that we worked on. We always collaborated with a group that was a real expert in that area.

Plum Island Animal Disease Center, Long Island, New York

Hughes: Were the Plum Island scientists competent?

Yansura: They were competent scientists, except that their labs were out of the fifties. They were held back by being in an underfunded federal lab. All the equipment was old, and it was very bureaucratic. It was very interesting working with them. They were very nice and competent scientists, but they had these drawbacks in their situation.

Hughes: Why was the federal government supporting work on a vaccine which wasn't relevant in this country? Because we are connected to the rest of the world?

Yansura: Yes. And if foot-and-mouth broke out in the U.S. we would need a vaccine.

Hughes: Was there an existing vaccine?

Yansura: Yes, there were vaccines out there. I think Burroughs Wellcome was one of the companies that made vaccines for foot-and-mouth. They sold them in South America and Europe and so forth. They made the vaccine from killed virus.

Hughes: How effective was it?

Yansura: It was apparently not very effective.

Now we really didn't know much about foot-and-mouth vaccines, so we counted on the scientists at this Plum Island USDA facility. What they told us was that the vaccines out there were not particularly effective in that they wouldn't give complete protection, and they would sometimes give rise to new outbreaks because the virus was not totally killed. It is an RNA virus which is constantly evolving, much like the AIDS virus. It is hard to get a good vaccine for an antigen that's constantly evolving.

Hughes: Did you go to Plum Island to work?

Yansura: Yes, we would go for one to two weeks at a time, and we would pack our equipment in boxes and take it there. It took days of organizing, and then it would take all day to fly there. We would fly to New York City, and then we'd have to drive the full length of Long Island, and then we would rent an apartment there.

Hughes: And you went by boat to Plum Island?

Yansura: We would go by boat, which had a regular schedule. Maybe 150 people would get on the ferryboat every morning, and we would get on with them, and it would take a half-an-hour or so to get to Plum Island. Once you were on the island you were sort of a prisoner. They drove you to the lab. You always had to have someone with you. The lab was a P4 lab, and so there were no windows, and you couldn't leave. It was psychologically hard to work that way.

Hughes: How did you do under those circumstances?

Yansura: We had our limits, so we would only go for one to two weeks, and that was about all we could stand because you were pretty much locked in the lab. You could go from the lab to the bathroom but you couldn't go anywhere else. Someone would have to come and walk you to the lunchroom.

Hughes: What was the idea of always being accompanied?

Yansura: It was for security. They not only studied foot-and-mouth disease but a number of other animal-virus diseases. It was essentially a quarantine island. They wanted to keep all those viruses contained.

Hughes: What was the procedure when you left in the evening?

Yansura: Anything that you took into the lab you couldn't take out, except for your body. You'd shower coming out. You'd wear little white uniforms they would provide for you. In some ways it was a great experience.

Resistance to Adopting Recombinant DNA Technology

Hughes: Was there an attempt to integrate recombinant DNA into the Plum Island procedure on a permanent basis?

Yansura: There actually was. Towards the end of the project they hired a scientist--I believe her name was Betty Robertson--to bring in the new technology. You had the feeling that all the scientists there at the time were too fixed in their ways to try a new technology.

Hughes: With you working right there, it could be seen as a golden opportunity to learn a new technique.

Yansura: Yes, it could be, and surprisingly nobody wanted to do that. They sort of left us alone.

Hughes: Did you interact with them?

Yansura: Yes, we interacted with them quite a bit, even socially. We went to their homes for dinner and so forth. They were very happy to be collaborating in this manner. It was their moment of glory. Eventually we got a vaccine to work with one strain, and the Secretary of the USDA made the announcement. It was a great period for them to get some applause. But they did not grasp the new technology.

Pharmaceutical companies had different ways of acquiring new technology, but it wasn't necessarily to adopt it in-house. However, some of the large pharmaceutical companies did try to bring in the technology. Nowadays they all have it, of course. It seemed difficult for certain groups to bring in the technology. Certainly for the USDA group, it was very difficult. They had to hire someone special, someone newly out of school, to bring it in because they couldn't convince anybody in-house to try it.

NIH Guidelines for Recombinant DNA Research

Hughes: How many other P4 labs do we have in this country? There's one at Fort Detrick, right?

- Yansura: I'm not sure how many there are or where they are. Most of them are under the Department of Defense. There are not many. When the insulin work was going on, Wally Gilbert's group had to find a P4 facility to do their work, and they had to go to England. That's how sparse these labs are here.
- Hughes: The UCSF group went to France. That brings up the question of the NIH guidelines. Do you remember the year the foot-and-mouth project began?
- Yansura: My guess is about 1980. The guidelines were relaxed in terms of being able to clone human DNA on the bench. For certain viruses you had to use a P4 facility, and for this virus, the RNA was somewhat infectious. It couldn't leave the island, so we had to go there.
- Hughes: What about safety precautions with hepatitis B?
- Yansura: [pause] I don't recall any problem cloning that. It could be because the regulations were relaxed by then.

No Danger of Infection

- Hughes: How worried were you about your own safety? It was an infectious agent after all.
- Yansura: We started with DNA.
- Hughes: So you never had to deal with the virus itself?
- Yansura: Right.
- Hughes: Where did the DNA come from?
- Yansura: I can't recall exactly where it came from.
- Hughes: From outside Genentech?
- Yansura: It came from outside Genentech.
- Hughes: You bought it?
- Yansura: No, we had collaborations. I think we had a collaboration with someone at the Wistar Institute. I can't recall his name. So we got samples of DNA. You could take the virus, get rid of all the protein by extracting with phenol, and just have the DNA. The DNA in general was not known to be infectious.

International Minerals

Hughes: International Minerals and Chemical Corporation was going to produce the foot-and-mouth vaccine, right?

Yansura: Yes. We had started this collaboration with USDA on Plum Island but we needed someone to actually produce the vaccine and sell it. You almost have to be in the business of dealing with international agriculture to go out there and sell this thing. We needed to have salesmen going to South America and Europe to sell this animal vaccine. So we were always looking for someone to buy this vaccine from us. In other words, we would produce it, do all the recombinant work; another company would take it over, just like Eli Lilly took over the insulin project. And then we'd get royalties.

International Minerals Corporation made an offer, so we worked with them for a while. It seemed like a poor match, even from the title. We had a hard time figuring out how this vaccine fit into their business plan. We also had an agreement with Bayer in Germany for a while. I think a lot of companies at that time simply wanted to get in on the recombinant DNA business and activities just so they were part of something new and not left out.

Hughes: Why didn't the partnership work very well?

Yansura: We started with a laboratory strain of foot-and-mouth virus; it was called A12. There were several major groups and subgroups of the virus, based on their surface proteins. It was a virus that was constantly mutating, much like influenza. So you maybe had a vaccine for a while, but then it was going to evolve, and then you would have to change the vaccine again to keep up with it. Our strategy was: As a new virus came out, we'll quickly sequence it, make the new antigen, and try to keep on top of it, much like way the influenza vaccine is handled now. So that was our plan.

We made the protein in *E. coli*. We didn't refold it; it was aggregated in inclusion bodies. We purified that protein and injected it into cows and pigs. There's nothing wrong with that idea since the native epitopes should probably be there anyway. They challenged the animals with the live virus and it protected them. So it worked exactly how we hoped it would. We got a lot of coverage. That was our highlight of the project. For us it was exciting. But I think it was almost more exciting for the group on Plum Island. They really got a lot of mileage out of that. We wrote the paper and submitted it to *Science*, and it got selected for the best paper of the year. We received the AAAS Newcomb-Cleveland award and got to split 5,000 dollars. We also received bronze medals at a ceremony which happened to be at one of their meetings in Detroit. My parents and sisters were able to see the ceremony. In terms of publicity for the Plum Island USDA facility, it was a great occasion. They just loved it.

But then reality set in. We had a laboratory strain that worked; now let's do some field strains. We started working on the field strains active in South America and Europe. We would go to Plum Island and clone out the surface protein genes called VP3. We'd

bring back the plasmid DNA to Genentech and make protein. It turns out that the [field] strains didn't work as well. Some of them worked a little bit, and some didn't work at all.

Hughes: Because the virus was so variable?

Yansura: Nobody ever figured it out. One possibility is that the challenge virus had evolved with the vaccine. Eventually the project died.

Hughes: To your sorrow?

Yansura: No. I was not that emotionally attached to most projects, because there's always another project or should I say another protein. You like to see what you're working on make it, that's true, and you feel a sense of pride when a product is out there that you worked on -- insulin, growth hormone, whatever. This was an animal vaccine, and it was going to be sold in South America or Europe. It was more removed. We had gone to Plum Island many, many times, and we were actually quite sick of going there.

Genentech's Original Mission Narrows to Human Pharmaceuticals

Hughes: I don't recall ever having read that animal disease was part of Genentech's mission.

Yansura: The original mission was everything--anything that we could do with recombinant DNA. We had projects to make silk and rubber, as well as animal products, proteins, and human pharmaceuticals. We were open to anything. Eventually that changed, and we spun off, for example, industrial enzymes to a company called Genencor. Then eventually we got rid of our animal products division.

Hughes: Were you part of the animal products division when you worked on foot-and-mouth?

Yansura: No. There wasn't really any distinction; you were just working on whatever project it was. But eventually Bob Swanson realized that we had to focus, that to be working on industrial enzymes and human pharmaceuticals was too broad.

Hughes: When do you think the narrower focus came into play?

Yansura: My guess is in the early eighties.

Hughes: So at the time of the IPO, which was in the fall of 1980, Genentech was still a pretty diffuse company?

Yansura: At that time I believe everything that we were working on was geared towards human pharmaceuticals. But we had in the planning stage to go into animal products--interferons for cows, bovine growth hormone (which actually became successful), and then all of these industrial enzymes--subtilisin for detergents for example. We had ideas to make silk and rubber in bacteria, but nobody knew quite how that would work. We had all these

ideas, and we were expanding from just human insulin and growth hormone into all those other ideas. We thought, yes, we'll just do everything.

Hughes: Was it Swanson that was particularly behind this comprehensive approach?

Yansura: Yes, he had to bring in money to keep us going. So the business development group and Swanson would try to strike up deals just to bring in enough money, and we would work on the project to keep us going. Swanson maybe thought we needed enough money just until we got growth hormone on the market.

Hughes: Did it work that way? Growth hormone of course did become a Genentech product, but did it generate enough money that you could abandon a scatter-shot approach to your research projects? Did the success of growth hormone change that strategy?

Yansura: Yes, eventually it did. We were getting some money from Eli Lilly for insulin and from other companies and venture capitalists. As Bob would say, "To keep the doors open and your paychecks coming, we've got to do all of this other work." It was clear at the time that many of these projects we were doing were just to keep the money coming in, to keep us going. Eventually human growth hormone did make us quite a bit of money, way more than we thought, and I think that was very critical for the early years of Genentech.

Hughes: When did growth hormone begin to be lucrative for Genentech?

Yansura: There was another source of growth hormone at the time, and it was isolated from cadavers. Somehow there was contamination of the growth hormone; some of the cadavers had prion diseases which eventually infected some children. When that information came out, that was the end of cadaver-derived growth hormone, and that opened up everything to recombinant growth hormone.

Hughes: We'll save the rest of that story until the trial is over.

Early Company Culture

Hughes: Please comment on the culture of the company in the early days, and how it might have been different from that of other biotech companies that eventually grew up.

Yansura: When the company was very young, everybody in the company was very young. That set the tone in a lot of ways. There were a lot of pranks on different people. Some of the work was somewhat boring so you wanted to break it up. We spent a lot of hours here during the early days, the insulin days, and you would get bored; you needed a break. Dave Goeddel was very good at this. He would work really hard, and then he would need a break and think of some prank to play on somebody. So that kind of stuff was going on all of the time.

Hughes: Can you describe one particularly memorable prank?

Yansura: We would have parties every Friday. That was the culture at Genentech. When I first came here, Bob Swanson would make sure that there was beer in the little fridge so that anytime you wanted a beer you could go out and get a beer. People didn't do that too often because once you have a beer you don't feel like working very much. But on Friday afternoons we would say, "Okay, let's take a break." Everybody would cut off from their work at five, and we would have a small party, just chips and beer and so forth. This became a tradition at Genentech, and to this day we still have these parties every Friday. They're called ho-ho's.

In the early eighties, when we had several hundred people at Genentech, Dave Goeddel and a few others decided that they were going to sponsor a chili ho-ho. They sat around and watched everybody eating this chili and telling them how good it was. It didn't get out until the next Monday that it was made with dog food.

Hughes: What reaction did he get?

Yansura: See, at that time you could get away with things like that. Nowadays you wouldn't think of doing something like that; someone might sue you.

In the lab we had a little hoop and a Nerf basketball. Every once in a while Tom Kiley from legal would come in, and there would be a little game of basketball in the lab. So there was a lot of that going on. Everybody was fairly young, in their twenties, early thirties.

Hughes: What happened to the culture as the company grew?

Yansura: As the company grew of course the average age grew also. People got married and started families and just naturally become more serious.

Hughes: Do you feel that in yourself?

Yansura: Yes. I don't have the urge to do those early pranks any more. I'm more interested in the intellectual things at work now.

Hughes: How is it to work here now?

Yansura: It's very corporate.

Hughes: What comes to your mind when you say that?

Yansura: Let me restate that: Genentech's much more corporate than it was early on. Early on everybody was like a teenager. Nowadays it's certainly more mature. On the other hand, it's still very loose. You can wear anything you want, and the hours are very flexible. So there's a lot of freedom, and people like it that way.

Hughes: Inevitably, with much larger numbers, the original collegiality must get diluted. There must be lots of people that you don't know at all.

- Yansura: Yes, that's true. I don't go to these Friday ho-ho's anymore because I don't know anybody. People in research generally don't go anymore.
- Hughes: If the scientists rarely mix with the rest, doesn't that affect the cohesion of the company?
- Yansura: Well, yes. Most people in research don't feel that close to the other divisions in the company. There's QC or Quality Control and Manufacturing, and we have no idea what they do. The original culture of Genentech has somewhat stayed within research in that it's still fairly loose here. Yet there are not the pranks, and people are more serious. People do get fired here in research--much more often than in other parts in the company.
- Hughes: Why is that?
- Yansura: In most parts of the company you have to do something wrong or be incompetent to be let go. In research, they may decide that Genentech is not going to go any further in your particular area, and your job is lost.
- Hughes: And the skills nowadays are so specialized that you can't move a scientist from a dropped project and put him or her on another one?
- Yansura: That's correct. In the early days we did recombinant DNA and protein chemistry, and everybody could do a little bit of everything. Nowadays it's very specific.

Human Insulin

[Interview 3: December 7, 2001] ##

Synthetic DNA and Cloning

- Hughes: What had already been done on the insulin project when you arrived on the scene?
- Yansura: The synthetic DNA was essentially made.
- Hughes: Was that all made by Crea? What did Itakura contribute to the DNA synthesis of insulin?
- Yansura: I have always had a hard time figuring out what Itakura really did during this period. I assume that he had some role in the synthetic DNA as he was in charge of this part. He played a major role in designing the insulin gene early on. But Roberta Crea was the lead person actually getting the synthesis done.
- Hughes: Were you receiving samples from City of Hope, or had all the transfer of material been accomplished by the time you got here?

Yansura: Most of it. There were still some fragments that maybe had to be remade. I think Itakura ran the synthetic DNA lab, and it was Roberto Crea who worked with a small group to actually do the work. So they had them cranking out the DNA, which had to be done before putting the fragments together into the full gene.

When I came, Dennis Kleid and Dave Goeddel had put together at least one of the chains. I think the A-chain was made and cloned as one piece. And the B-chain, because it was a little bigger, was cloned in two pieces. So you had the option of combining the correct pieces. There were so many mistakes in the synthetic DNA back then that to make something like this you're going to have to sequence a few DNA chains and figure out where the mistakes are and maybe recombine pieces.

Hughes: So you'd snip out the mistakes and link the correct sequences?

Yansura: No. You couldn't easily correct it at that time. Once you cloned a fragment you might have to sequence several of them before you found one that was correct.

Hughes: Is that because DNA synthetic methods at that time were not very accurate?

Yansura: Yes, that's part of the reason. But even today when we order synthetic DNA and use it to make gene fragments, we always realize that wherever the synthetic DNA lies, that's where most of the mistakes are.

Hughes: You look there first for mistakes?

Yansura: Yes, and you try to limit your use of it.

Hughes: Wasn't there a reversed codon in the insulin project?

Yansura: There was a mistake, I believe, when they cloned the B-chain. Dennis Kleid had a habit of not labeling things and got them mixed up. I don't think there was any other major mistake.

Hughes: When you decided to clone the B-chain in two different steps was that a guess that the whole chain was probably too big for *E. coli*?

Yansura: Well, first of all, it certainly wasn't too big. It's actually very small for *E. coli*. I believe that Art Riggs and Itakura had designed the DNA early on. They had gone through a lot of effort to put in preferred *E. coli* codons. But they also realized that there were going to be mistakes, and the longer the gene is, the more likely you will have a mistake in there. If the gene is really long you may have to sequence a hundred genes before you find the correct one, but not if you put a restriction site right in the middle so you can mix and match different correct pieces. Then your luck goes up tremendously. So they realized that, and they engineered in a HindIII restriction site, I believe it was.

The B-C-A Approach

Hughes: What did you immediately start doing after you arrived?

Yansura: I had never cloned anything. I had worked with synthetic DNA, and the next step is to ligate it into a plasmid and then transform. So there wasn't too much more to learn from what I had done at the University of Colorado.

I started out on an alternative plan to make insulin. There were two approaches. One approach was to make A-chain separately and B-chain separately and combine the two. It was known that you could take bovine or pig insulin, take the chains apart, and recombine them. So that step had been done previously and was known to work. There was also an effort to make a mini C chain. Normally, insulin is made with a C chain, which connects the A and B chains and tethers the two together so that they fold efficiently together. The C chain is later cleaved out. So there was synthetic DNA to make this so-called mini C gene. That's what I started to work on.

Hughes: Is that the B-C-A project?

Yansura: Yes, that's the B-C-A project.

Hughes: So you were the C?

Yansura: Yes, I was working on the C, although it was attached to the A and B genes.

Hughes: How did that go?

Yansura: My recollection is that it went together okay. I think there was one problem getting part of it to ligate together. Eventually, I believe we had to get one DNA fragment remade. The B-C-A project was considered as a backup. If the two-chains approach didn't work, this would be the backup. The whole scheme at the time was to have multiple backups. Dennis Kleid was always doing the backup for Dave Goeddel's experiment if it failed. So there was redundancy. We knew we were in a race, and we knew we would have to be first to survive as a company.

Pressing Need to Beat the Competition

Hughes: Of all the competing groups, you at Genentech were the most dependent on the success of this research.

Yansura: That's correct.

Hughes: I assume you're thinking of the Gilbert group at Harvard and the Rutter-Goodman group at UCSF.

Yansura: That's correct.

Hughes: Both of course are in academia. They don't have a company whose survival depends on the success of this research.

Yansura: Yes, they would still have their jobs at the university. Although surprisingly, Biogen was started at that time to have a place for the insulin project to go, and they still survived even though they didn't win the race for human insulin. They were the first to clone murine--or rat? Maybe it was rat proinsulin. That came out, and it gave them a psychological boost in that they had been able to pull that off. I think they had expressed some of the protein in a funny or should I say not very useful form.

Wally Gilbert's group was the first to get the rat preproinsulin. Somehow that ended up on the news. We were all very worried about that for a while. But then as we got more information we learned that it was just the rat insulin gene, so we were relieved.

Hughes: Were you during the insulin project very conscious of your competitors, as opposed to just getting on with the research?

Yansura: We worried every day.

Hughes: How much were you working?

Yansura: Well, I asked my wife, Patricia, and she says it was pretty late. I was getting home at ten or eleven at night.

Hughes: And starting when?

Yansura: Eight or nine.

Hughes: And that was pretty consistent for a while?

Yansura: It was pretty consistent for months. I took off Sunday. Dave Goeddel would make you feel guilty. He didn't come down too hard on me.

Hughes: Were you used to working that hard at Colorado?

Yansura: [pause] No, we never worked that hard at Colorado.

Hughes: To work that hard, one would have to be in a race; one would have to have competitors. Otherwise why bother?

Yansura: Right, why bother? There was something to be said for the competition in that it was exciting, whereas the work at Colorado many times got very boring because everything was slow. Here things moved really fast. We were never bored.

The Somatostatin Project As a Model

- Hughes: What were your major concerns in the insulin project? The parts that you were not at all sure would work?
- Yansura: Well, we knew that we could put together the gene, and that we could eventually get it constructed right and transformed into the bacteria. We weren't as sure about expressing the protein. At that time, there was a fair amount of knowledge in terms of how *E. coli* synthesizes protein. But we were putting in a totally synthetic gene, and it was from a different organism, and nobody really knew what the rules were. Nonetheless, because somatostatin had been expressed, and the main thrust of our insulin work was to use a repeat of what was done for somatostatin, we felt reasonably sure that we would be able to make both protein chains.
- Hughes: How much did you have to modify the expression apparatus for the insulin gene?
- Yansura: We didn't manipulate it at all. We used exactly what was used for somatostatin because that was the surest thing. Each insulin chain is a little bigger than somatostatin but not terribly bigger. So we figured that we could repeat what was done with somatostatin. Somatostatin was made as a fusion with the *E. coli* β -galactosidase protein, which is a humongous protein. The feeling at the time was that you had all this natural *E. coli* protein being made, and you were just going to slip in this little human piece at the end, and the *E. coli* probably wouldn't complain too much.
- Hughes: What about the folding of the peptide once it was expressed? What did you think was going to happen there?
- Yansura: Because it was made as a fusion with beta-gal, then you had to cleave it off before you folded it. You had to cleave it with cyanogen bromide, which is toxic. That cleavage was used for somatostatin, and it's a reasonably good cleavage. It destroys the whole protein and attacks all the methionines. There are no methionines in the insulin A and B chains, and methionines were strategically introduced at the beginning of each chain. So we knew that that would probably work.

Finding and Fusing the A- and B-Chains

- Yansura: Then you had to fish out the little peptide A chain and B chain among all this other beta-gal stuff and some *E. coli* proteins because we weren't equipped to deal with a lot of protein purification and manipulation. A really tenuous part of the insulin project was getting the two chains out of the fusion protein, getting them to come together, and then once they came together we had a way to detect them. There was a radioimmunoassay for insulin at the time, a commercial kit.
- Hughes: That was lucky.

- Yansura: In some ways it was, but insulin was such a well-known basic protein in medicine that there were assays for it.
- Hughes: Was this fusion of the two chains a natural phenomenon? How did that part of it work?
- Yansura: Well, there was nothing natural about it. [laughter]
- Hughes: How did you join the chains? You used your C chain?
- Yansura: The chains were first chemically modified by adding sulfonate groups to the cysteines. Then the chains were simply mixed together and the cysteines after shuffling around form the correct disulfides. At least to some degree.
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- Hughes: Did Herb Boyer actively participate?
- Yansura: Hardly at all. He was sort of a father figure, and yet he wanted to keep his distance so that the boys could do it themselves. That's sort of the way it worked.
- Hughes: Was it his suggestion to use the EcoRI site, or was that obvious to the rest of you?
- Yansura: Well, the EcoRI site was used to make somatostatin. There was an EcoRI site in the beta-gal gene near the end, and we knew what amino acid frame it was in. So you could cut it at that site and you knew exactly what the reading frame would be, what the DNA sequence would look like. That was known very early on, to make the somatostatin fusion. We simply used that same EcoRI site for insulin. In fact, there weren't that many restriction sites available to use at that time; there was maybe half-a-dozen.

Sequencing

- Hughes: Did you do the cloning?
- Yansura: Not on the individual A and B chains, but I did the cloning for the B-C-A insulin. I also worked on the DNA sequencing of B chain. Dave Goeddel had cloned the genes, but you needed to verify the sequence, so I sequenced the B-chain.
- Hughes: How tedious was the sequencing in that era?
- Yansura: Compared to today's standards it was an art. It was very temperamental and touchy. It was a fairly new technique that Wally Gilbert and Allan Maxam had come out with.
- Hughes: Was the Sanger method around and about yet?
- Yansura: We had actually used this two dimensional sequencing method at Colorado. That was even more primitive.

- Hughes: So you could get better results with Maxam-Gilbert?
- Yansura: Yes, much better. And you could read a lot farther. It still was temperamental.
- Hughes: Did that mean you had to repeat things?
- Yansura: Well, the way I recall the sequencing is that it went fairly straightforwardly. You generally had to sequence in multiple ways, frontwards and backwards, so that you had both DNA strands covered. I think it went fairly well. Later Bob Swanson used copies of the x-ray films from the sequencing gels as a demonstration or proof of our work, because there was no easy way to visualize a synthetic gene. So he framed the DNA-sequence ladders for the A-chain and B-chain.
- Hughes: Is that still around?
- Yansura: It probably is. I still have my original sequencing gel films.
- Hughes: Was City of Hope doing anything other than supplying the synthetic DNA?
- Yansura: Well, yes. They had played a major part in the start of the project in that they had designed the genes and put in preferred codons, and they had designed the HindIII restriction site in the middle of the B-chain. So all that had been done early on. I'm not sure what part Boyer played in that. Riggs and Itakura got the patent on the gene design, so they obviously played a major role.
- Hughes: What did Herb Heyneker do in terms of insulin?
- Yansura: Heyneker played a major role in the earlier somatostatin work. He had gone through the process; he knew exactly what to expect. He showed up a little during the insulin project. He wasn't physically available [at Genentech] for doing much work.
- Hughes: Was he in Holland at that point?
- Yansura: My recollection is yes, he was living in Holland with his family, and he was here just for a short period during the insulin project.
- Hughes: Did he give you advice based on his experience with the somatostatin project?
- Yansura: [pause] It was fairly clear how to put the gene together and transform it into the bacteria and get the protein expressed. The harder part was isolating the A and B chains from the mess of protein after the cyanogen bromide cleavage.
- Hughes: How did you do that?
- Yansura: Dave Goeddel did most of it down at City of Hope. They were better equipped down there. They had an HPLC to do some purification.
- Hughes: What's HPLC?

Yansura: High-pressure liquid chromatography. You can put proteins and peptides in there, purify, or separate them. It's basically chromatography. So most of the protein work was done at the City of Hope, at least up to the point where we had first made insulin and announced it. After the announcement and we signed an agreement with Lilly, we did a lot more protein work with insulin, but it was done at Genentech.

Expressing Human Insulin: An Anticlimax

Hughes: Did you first hear about your success from somebody contacting you from the City of Hope?

Yansura: Yes, Dave Goeddel called.

Hughes: Was getting insulin a big deal?

Yansura: Dennis Kleid did go down there somewhat, but most of the time it was Dave Goeddel doing the work. They were trying to work out the purification and the recombination reaction. Art Riggs was helping Dave Goeddel, and Dave Goeddel was working twenty hours a day and just taking out time to sleep. Art Riggs was living a more normal life at this time.

Hughes: And driving Dave crazy because of that?

Yansura: And driving Dave crazy. We heard a lot of those stories later.

Hughes: I heard that Riggs was a very methodical scientist. Were Riggs's and Goeddel's styles of doing science very different?

Yansura: Yes, they appeared to be different. As you said, Riggs was methodical, went one step at a time; Dave was, "Let's try for a home run first, and then we'll try for third base after that, and then second. We'll have all this going on at once, and then we'll see what's the best we can do." So he had multiple things going on at the same time. [animal clock chimes]

Hughes: The way you tell this, it comes across to me that it wasn't a particularly spectacular moment when you got that call from Dave Goeddel saying that they had human insulin.

Yansura: Yes, it was somewhat anticlimactic. First of all, it seemed like it took a long time, maybe a month, for Dave to be down there playing with this. You may get a positive result with the RIA, the radioimmunoassay, but then you want to be sure, because you do get some false positives. So even when you do get a positive result and you think it's real, there's always in the back of your mind, "Jesus, is this really real?"

But we always felt that it had to work because all the groundwork was laid beforehand. Insulin, at least bovine or porcine insulin, you can take the two chains, spread them apart, and put them back together and get wild-type insulin out. That was known.

The cleavage of the fusion protein was fairly clear. So everything was known; it should work. You could see this huge protein on an SDS gel which was presumably the β -gal fusion, and stain it and see that it was there. I don't think there was any way we could know for sure that the insulin chains were on the end. So you just had to have faith that they were there.

Hughes: And you did have faith?

Yansura: [pause] Well, yes, I guess we had to have faith that they were there. If you think too hard about a lot of these things, you can talk yourself out of doing the whole experiment because there are so many things that can go wrong.

Press Conference, September 6, 1978

Hughes: There was a press conference at City of Hope. You and everybody that worked on insulin were there, is that not correct?

Yansura: Yes. Bob Swanson wanted to make this a big thing because he wanted to push Eli Lilly's hand to sign an agreement. Up to that point, we had all thought that Swanson already had an agreement with Eli Lilly. But Swanson also wanted to make a big splash because he was going to have to attract more capital. We had never thought about a press conference. It was pretty exciting to go down there.

Hughes: Tell me what it was like.

Yansura: We all got dressed up. It's not like any of us had been to a press conference before. It was clear that Bob didn't want too many people talking because he wanted to control the message.

Hughes: He hadn't said that in so many words? You picked it up?

Yansura: We picked it up. We did say something. I remember someone asking me something, and I said something about bringing together different existing technologies.

Hughes: Were you all informally milling around, and the journalists came up to you?

Yansura: No, we were on an elevated platform, a stage. There were a number of reporters below us who had cameras.

Hughes: Were you surprised at the attention?

Yansura: [pause] I don't know if I was surprised. It was exciting though. I was thinking, well, this is fun to do at least once in your life.

After that we all went to lunch at a Mexican restaurant. I hadn't met Art Riggs or Keiichi Itakura before. We had a nice time. We flew back in the afternoon. We got off the plane and walking down the terminal we could see that we made the headlines. That was when it really sunk in. We thought we were doing something important but not quite sure how the outside world would react. And that headline was the proof.

Hughes: There was some static around the subject of making a public announcement about insulin before a paper had been published. Do you remember any specific instances where that criticism was expressed?

Yansura: I don't know who exactly expressed it, but the criticism came out fairly quickly from the academic community. I don't think anybody here really cared. We knew that we were playing a different game, and we knew we had to do this to attract capital. To keep the company going, we had to do it. Everybody felt that we had really accomplished what we had said in the press conference: We had made human insulin in bacteria.

There was some criticism that the scientific community hadn't peer reviewed what we had done, and all that. To some extent that was true, although we had a paper written up, but of course it took months to get it published. It's just one of those chances that you take. We had no choice. We didn't worry about it too much.

Hughes: Another criticism was that no evidence was presented that the insulin was biologically active.

Yansura: Yes, that's true. That would have been a very difficult test because we had made so little insulin that I don't know if we could have found an organism that was small enough.
[laughter]

Hughes: Did you worry about it?

Yansura: No. I suppose the reason is, I think like a chemist. If it looks like a duck and quacks like a duck, it's a duck. I don't worry about whether it came from Mars or Earth. We had detected insulin with a radioimmunoassay. People knew that you have to have the correct structure for the antibody to bind.

Hughes: So the positive assay result was enough evidence for somebody looking for chemical proof of insulin's existence? You didn't need to have evidence of biological activity to be convinced that you had insulin?

Yansura: Right. The reason is, people had taken insulin apart and put it back together and shown that you get authentic insulin. That was published and was part of the scientific literature. We were doing the same thing. We were just getting our chains from a different source.

Extracting Insulin from the Bacteria

- Hughes: In contrast to somatostatin, insulin was not expressed into the medium; you had to break apart the *E. coli* to get the insulin?
- Yansura: Actually, for both proteins you had to break apart the *E. coli*.
- Hughes: Was there a strategy involved?
- Yansura: Well, normally insulin is secreted out of the mammalian cell. It's translated into a protein and folded. The C chain is cleaved out, and eventually it gets dumped out of the cell into our body's circulation system. That's considered the classical secretion method of expressing a protein. *E. coli* in some ways can do a similar thing. It will secrete proteins into the periplasm. The signal sequence gets cleaved off at that point, just like mammalian proteins do it. But that technology wasn't available at the time. Nobody knew how to get things secreted. It was known that there were signal sequences on proteins that are involved in getting a protein secreted. It represented another level of the complexity that Wally Gilbert thought that he could do. He thought he could express insulin in *E. coli* and it would automatically get secreted and then processed and so forth. But he was wrong. It's not to say that it can't be done. In fact, nowadays we can secrete proteins into the *E. coli* periplasm and we can get them folded. But it's very difficult, and back then it was simply impossible.
- Hughes: Periplasm means around the perimeter of the cell membrane?
- Yansura: It turns out that *E. coli* has two membranes, an inner membrane, which is similar to a mammalian cell membrane. Then it has a little space and fluid called the periplasm, and then there's the cell wall and outer membrane.
- Hughes: And you wanted to get it into that space?
- Yansura: Well, no, we knew that was way too complicated.
- Hughes: But you did know that that's the way it is done in nature?
- Yansura: We knew that mammalian cells secrete proteins through their membrane, and at that point the signal sequence gets cleaved off. We didn't have any signal sequence on our insulin. We attached it instead to beta-gal, and we knew that beta-gal was a cytoplasmic protein because it accumulates in the cytoplasm. It's much simpler to express a protein that way than to try to express it as a protein and get it transferred out through a membrane.
- Hughes: And then you just spun down the bacteria?
- Yansura: Yes, you spin down the bacteria, and your protein is in the cell. Then you break open the cell. I can't remember if the beta-gal fusion protein was soluble or not. I vaguely recall that it was insoluble. Being insoluble is another advantage because the insulin A and B

chains are fairly small; they're not really stable to proteases in the cell. Had we tried to make it that way, I'm sure we would have lost everything.

Hughes: Genentech had had that experience when somatostatin was expressed but in such small quantities that enzymes were chewing it up before it was detectable. I remember Swanson telling me that his heart sank because he thought the technology wasn't working.

Yansura: Yes, and even today proteins that are small almost never survive. The way to make them is to attach them to a bigger protein, or else to make the protein at a very high level where it comes out of solution. We use that approach today to make proteins that are unstable.

Hughes: It's amazing to think of these bacteria dealing with weird proteins that they've never seen before.

Yansura: Surprisingly, because I've been expressing proteins in *E. coli* for twenty-three years now, very few of them are toxic. *E. coli* makes them, and if they fold right and they're soluble, some stay around and are active; some get degraded. A lot of them we make at very high levels, and they aggregate and come out of solution. These proteins end up in refractile bodies. Nobody's totally sure what these are, but under a microscope they're little dark, dense particles inside the cell. Apparently, all the protein simply gets pushed together into a little garbage can.

Hughes: So *E. coli* thinks it's gotten rid of them?

Yansura: Well, not totally, because you can tell *E. coli* is not happy. When the Eli Lilly group started to play with the *E. coli* making insulin, they published a paper, I believe it was in *Science*, where they talked about these refractile bodies. When the bacteria make these refractile bodies, they get really long and lose their ability to divide normally. A lot of them are half-dead or dead already, but that's okay.

Hughes: They're producing insulin.

Yansura: Yes.

The Contract with Eli Lilly

Hughes: Tell me more about the contract with Eli Lilly that hadn't yet happened when the first scientists joined Genentech, even though the scientists thought it had. Presumably Eli Lilly was saying to Swanson, "No contract until we see that you can actually produce insulin?"

Yansura: That's apparently the way it happened. We were under the impression that Bob Swanson already had this deal. I thought it was written down and signed and all that. We found out later that it wasn't. We did, apparently, sign the contract very soon after the press conference. Once that was signed, we felt we were in reasonably good shape because we

had a source of money coming in. Dave Goeddel would talk about how many months of salary we had left unless we did this or that. That continued with the contract with Eli Lilly. We had benchmarks. Each time we reached a benchmark, we would get more money. Of course, that was our salaries. Ultimately, when the insulin got approved by the FDA, we would get royalties. So that would be additional money coming in at a regular pace. There's quite an incentive to keep that going.

Hughes: Do you remember scrambling to meet the benchmarks?

Yansura: Yes. There were more people at that time at Genentech. We had hired Mike Ross, who was a protein chemist. Yes, I recall these deadlines coming up. I remember having purified one of the chains. I was doing a little bit of protein chemistry at that time. I wasn't a particularly good protein chemist; it wasn't my training. I did it to help out. I didn't think it was very good work, so I stuck the protein prep in a freezer somewhere. I remember the deadline coming up. Mike Ross was scrambling to find some material to send to Eli Lilly so we could get the next payment. So I told him, "I have this prep but I didn't think it was very good." He was so happy to get it. It was enough to satisfy the next benchmark.

Hughes: Lucky that you squirreled it away.

Yansura: Things were very confused then. People were doing what they could.

Hughes: How much interaction was there with scientists at Eli Lilly?

Yansura: There were some regular contacts, mainly with the upper management of the project-- Irving Johnson is a name I recall.

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Hughes: Was Lilly responsible for the scale-up?

Yansura: Well, ultimately they had to be able to produce insulin themselves. There was a period where we were wondering whether it was really possible, and if it wasn't possible then we were all in this dream job that wasn't going to last. Our job in the industry was based on the fact that we could make proteins in a large enough scale to be able to sell them.

It always amazes me that Eli Lilly pulled off this scale-up in a reasonable amount of time. I say this because Eli Lilly was a traditional pharmaceutical company, and to think of them trying something brand new, trying to make a protein out of recombinant technology, always amazed me that they pulled it off. For a while I had thought that they would never be able to do this because they were not used to it, and they were set in their own old ways.

Hughes: Did Genentech hand over the clones to Lilly?

Yansura: Yes, we gave them the clones once the deal was signed.

Hughes: That was your obligation?

- Yansura: That was maybe the first benchmark, the easy one, because we had the plasmids. All we had to do was give them some more plasmid DNA, which was no big deal. They wanted us to show them that we could make more material. It took quite a while. Eventually, even though we gave them all of the original technology, it evolved within a year and it looked totally different from what we had come up with at our press conference. It was dramatically different.
- Hughes: Genentech hadn't handed over the technology to Eli Lilly because you had taken the technology a step further? Is that what you were saying?
- Yansura: No, that's not true. We gave them exactly everything we had. It's just that to go from what we had done at our press conference to making the stuff large scale and selling it was a big leap. That was a tremendous difference.
- Hughes: Eli Lilly took it from laboratory scale to commercial scale?
- Yansura: Yes, but we had to help them. We started off with these two chains made separately and combined them. Eventually, what we handed them was a continuous B-C-A construct. In fact, we handed them the human insulin cDNA gene. We had expressed it in *E. coli* at high levels as the B-C-A or proinsulin form. It turns out that a larger percent of the protein is now the actual thing you want at the end. With the large beta-gal fusions that we handed them initially, maybe 5 percent of that protein was actually insulin. The rest was just carrier protein. So we did give them a lot of improvements and a new promoter system. They still did quite a bit from there and eventually commercialized it.
- Hughes: Was Genentech trying to control the technology so that Eli Lilly could only use it in the production of insulin?
- Yansura: [pause] Well, I wouldn't say that we were trying to control it. We had filed patents whenever we could. It turned out that Eli Lilly used some of the insulin technology that we had given them to make human growth hormone. We eventually got into a legal fight over that and won.
- Hughes: Because the contract had restricted Lilly's use to insulin?
- Yansura: Yes. And they had used our insulin promoter to make human growth hormone.
- Hughes: What was your impression of the quality of the Lilly scientists?
- Yansura: We didn't have that much direct contact with them. The contract was in place; we would pass the materials, but ultimately they had to pull it off themselves. There must have been some tremendous incentive for them to do that. It probably was the fact that they were one of the major insulin producers in the world, certainly in the U.S., and if they wanted to maintain that position they had to commercialize recombinant human insulin.
- Hughes: At the time, many other pharmaceutical houses were taking a wait-and-see attitude towards adopting recombinant technology. What you're saying is that in a sense Eli Lilly

didn't have a choice if they were going to remain competitive in the insulin market? I assume insulin was their major product.

Yansura: They certainly had other products, but in the U.S. they were the major producers of porcine and bovine insulin. So they were the king of that market. If they wanted to maintain that position, they were going to have to come out with recombinant human insulin. There must have been pressure to do it. Part of the reason I say that is, as Genentech got more mature we got into the same mode, so parts of our company are now just as entrenched and not open to change as I thought Eli Lilly was. Nowadays, if Genentech wanted to make human insulin, we would have a hard time making it here. Certainly we could make it in research, but once we get out of research there's this entrenched bent, and there's this [attitude]--afraid to try new things.

For example, about ten years after insulin was out, we tried to commercialize a protein called relaxin. Relaxin is in the insulin family. It's fairly similar; it has a B chain, a C chain, and an A chain. It took us a long time to make that protein. We had to come up with an idea in research, and then we had to force it on the development side of the company to try something they weren't used to. Eventually they did it, but it was quite a struggle.

Hughes: It's ironic, isn't it? The more successful a company is, the larger it tends to get, and in that process loses some of its quick turnaround and innovative spirit. [laughs]

Yansura: Yes. The people that make the biggest splashes now, the real movers, are people that can take something radical through a system like Genentech's, which is a little conservative now and not able to change. People who push new things through are looked up to.

Hughes: Did the Lilly contract provide sufficient income?

Yansura: It was just enough to get started. It really wasn't until growth hormone came along that we had sufficient income at a steady rate, because growth hormone was something that we kept. We made it here and we sold it here. It turned out to be a bigger market than we thought. It was that money that really gave us stability. Every two to three months we'd get the benchmark payments from Lilly, but you can't live like that too long.

Hughes: Hand to mouth.

Yansura: Yes, and even when you get royalties you only get a few percent.

Hughes: I've read that young technology companies often make the mistake of selling the crown jewels, so to speak; they give away their prime technology because they're desperate for money.

Yansura: It was pretty clear that we wanted to give insulin away because we didn't have the ability to market it. There's no way we could have scaled it up and gone to the FDA with it. We only had a very small fermenter. You need a tremendous army of people to take a drug through the FDA. There was no way we could have done that. As for the new technology, we still had it, and we could use it again on the next protein that came along.

A Viable Commercial Product?

- Hughes: Did the fact that initially you had made so little insulin cause you to reconsider? Were you confident that you could make enough to have it become a viable commercial product?
- Yansura: Yes, we had our concerns. I know Dennis Kleid had some doubts. There were a few other people who had doubts that we could make enough. This was all new; we didn't know how much we had to make to make it a commercial success. The fusion protein was being made at maybe somewhere between 10 and 30 percent of the total cell protein, so you couldn't go up too much more from there and still have a cell that's going to grow. So it did seem that we couldn't go that route. Obviously we did it. We worked out what it takes: how much protein you have to make in a cell to make a viable process. Yes, there were doubts; there were doubts.
- Hughes: Understandably, there's nothing in Genentech's press announcement that indicates how little insulin had originally been made.
- Yansura: I recall worrying about that after the press conference. I didn't know how much Dave Goeddel made, but I assumed it wasn't a lot. Then I found out it was just barely detectable.

The Two Publications

- Hughes: Two papers were published on the insulin work, one on the cloning and expression, and one on the DNA synthetic component.¹ There's quite a lineup of authors. Do you remember any discussion of the order in which the authors should appear on the papers?
- Yansura: Yes, there were big fights. That's why there are two papers instead of one.
- Hughes: Tell me about it.
- Yansura: Well, Roberto Crea thought he should be first author. Itakura and Riggs were sort of the father figures. So we knew they would go at the end.
- Hughes: What about Dave Goeddel?

¹D.V. Goeddel, D.G. Kleid, F. Bolivar, H.L. Heyneker, D.G. Yansura, R. Crea, T. Hirose, A. Kraszewski, K. Itakura, A.D. Riggs, "Expression in *Escherichia coli* of chemically synthesized genes for human insulin," *Proceedings of the National Academy of Sciences [PNAS]* 1979, 76(1):106-10; R. Crea, A. Kraszewski, T. Hirose, K. Itakura, "Chemical Synthesis of Genes for Human Insulin," *PNAS* 1978, 75(12):5765-69.

- Yansura: Dave Goeddel thought he should be first. There were problems with Itakura also. So the way they resolved it was to write a separate paper for the synthesis of the DNA for the gene, and another paper for the cloning and expression.
- Hughes: Who wrote the papers?
- Yansura: I know Dave Goeddel wrote most of the cloning paper.
- Hughes: And probably Roberto wrote the other one?
- Yansura: I assume so.
- Hughes: Was there much reaction when the papers came out?
- Yansura: No. By then it was--
- Hughes: Old news.
- Yansura: It was old news. Interest peaked at the press conference.

Yansura's Work on Protein Purification and Proinsulin Expression

- Hughes: [pause] As soon as you had expressed insulin, Goeddel moved on to interferon and growth hormone?
- Yansura: Yes.
- Hughes: What about you?
- Yansura: I did some of the purification for one of the insulin chains, I believe it was the B-chain. I told you that story about putting it away. I eventually worked on expression of human proinsulin in *E. coli* without any leader or fusion. That apparently was the precursor of Lilly's ultimate process.
- Hughes: How were these things worked out? Goeddel would move on; you would get insulin expression working a bit better?
- Yansura: It was a fairly loose structure. I think it depended on people's interests, and Dave was interested in cloning. The rarer the gene or the harder it was to clone, that's what he wanted to do. My interests were in protein expression. That's pretty much my career at Genentech. I have done other things--cloning and vaccines a bit. But I veered towards protein expression. That's what I do today.

Hughes: Do you have any insight into the impact that insulin might have had in moving Genentech along to becoming a viable company? Did Swanson complement you scientists for giving him the selling point that he needed to attract more money?

Yansura: Well, yes. Maybe a week or so after the press conference, he called us individually into his office and gave us bonuses.

Intellectual Property

Hughes: How many patents were there on the insulin work?

Yansura: [pause] I don't know. My guess is there were ultimately maybe five to ten patents on it.

Hughes: And they did a good job of protecting Genentech's technology?

Yansura: Yes. The first patents were on making the gene. Then there was all these technologies that we came up with along the way which were also patented.

Hughes: Genentech has the reputation for being pretty aggressive in terms of its intellectual property. Was that true from the start?

Yansura: [pause] I don't know what you mean by aggressive.

Hughes: Genentech reputedly doesn't hold back when it comes to filing patents and suing companies that it thinks are infringing. Maybe I shouldn't have singled out Genentech; maybe aggressiveness regarding intellectual property is endemic to the biotech industry.

Yansura: Yes, everything is based on patents. You don't develop any new drug without patent coverage. In the early days, we had outside attorneys dealing with our patent stuff.

Hughes: And your feeling is what? That they did a pretty good job?

Yansura: Well, we hired a patent attorney. Walt Dreger, I think was his name. He did much of the early stuff. He died about five years ago. But eventually we had our own attorneys.

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Hughes: You scientists had to talk with the attorneys so that they could draw up a patent application, right?

Yansura: Yes. They tend to do a nice job. The patent attorneys here do a nice job of putting all this bulk into the patent. I don't know if you've ever seen a biotech patent. The first ten, twenty, thirty pages is what they call "boiler plate." [chuckles] It's almost the same from patent to patent. They talk about everything possible in biotechnology, and then right at the end you throw your results in there and you get the claims. They walk you through it.

- Hughes: The fact that biotechnology was a new field didn't throw them? I would think they might not have even known the vocabulary.
- Yansura: That's probably true. It was all fairly new. I assume the first patents were a lot of work for them.
- Hughes: People questioned whether patents in biotechnology were going to hold up.
- Yansura: Yes, right, and it turns out they did hold up and they were very valuable.
- Hughes: All right, we'll stop.

The Human Growth Hormone Project

[Interview 4: January 11, 2002] ##

More on DNA Synthesis

- Hughes: Do you remember at what stage the human growth hormone project was when you arrived at Genentech in June 1978?
- Yansura: It was more along the lines of an idea and something in the future. Initially, all the attention was on human insulin.
- Hughes: So growth hormone was on the business plan but nobody had done any lab work as of June?
- Yansura: Once the synthetic DNA was made for insulin, all the molecular biologists focused on making insulin. That was their total focus. But the synthetic DNA group, since it had already made the fragments for insulin, started to make part of the human growth hormone gene.
- Hughes: You're talking about the group at City of Hope?
- Yansura: Yes, that's correct. We hired Roberto Crea and his group.
- Hughes: But they were still located at City Hope in June of '78?
- Yansura: That is correct.
- Hughes: Do you remember when the synthetic DNA group started at Genentech?
- Yansura: My guess is that it was about the end of 1978, early 1979. We didn't have the labs for them set up, and they needed hoods and all that. So I'm sure that they were delayed coming to Genentech because the labs weren't complete.

- Hughes: Were the techniques that Roberto Crea and his group were using different than yours?
- Yansura: Yes, they were using a different method than the one we had used at the University of Colorado. There was a quantum leap in synthetic DNA about this time, or should I say a series of smaller leaps.
- Hughes: What were some of the leaps?
- Yansura: Probably the main improvement was how they connected or condensed the nucleotides.
- Hughes: They found a more efficient way of doing that?
- Yansura: Right--a more efficient way to couple nucleotides and at the same time prevent some of the side reactions. It was called the phosphotriester versus the older phosphodiester method. Eventually a machine did it all. It used to be all done by hand. You'd take one nucleotide in this vessel, and one nucleotide in this other vessel, and they were protected in a certain way, and then you would put them together with a condensing agent and let it go. Then you'd purify that. It was very laborious.
- Hughes: Did you find recombinant DNA technology less tedious?
- Yansura: That's what I was attracted to. At the University of Colorado we had made small fragments of DNA. Then we did some molecular biology where we would ligate these fragments together and make duplex DNA and eventually look at repressor binding. You got results in a couple hours or perhaps a few days, and it wasn't real tedious and boring.
- Hughes: I can't imagine Dave Goeddel having the patience to go through a long laborious process.
- Yansura: He didn't. [laughter]

Peter Seeburg's Arrival at Genentech

- Hughes: Seeburg came to Genentech at the end of 1978. Had you and others at Genentech been following the work that was going on at UCSF on growth hormone?
- Yansura: Yes, we were aware of what had been done, in particular Peter Seeburg's work. We were using that work to take off on our own--that was the plan.
- Hughes: As of sometime in 1978, Genentech was funding Seeburg's work at UCSF.
- Yansura: I wasn't aware of that.

Genentech's First Start-to-Finish Product

Yansura: Bob Swanson had the vision to see that insulin was just going to be a stepping stone, and growth hormone was going to take us the next step. He really wanted growth hormone. And it turned out he wanted to make that into Genentech's first product that we would not only develop here but actually produce at Genentech.

Hughes: Why did he choose human growth hormone as opposed to insulin?

Yansura: Human insulin is a humongous market because you have millions of Americans and people all over the world taking insulin every day. You'd need huge plants to make it. We didn't initially have that capability. The market for growth hormone was much smaller and very defined. It was the perfect case. Growth hormone derived from cadavers was always in short supply. We came out with a protein that was a copy of the original growth hormone that they got out of cadavers. The market was already there, and because it wasn't enormous we would be able to handle it.

Hughes: Swanson from the very early days talked in terms of a fully integrated pharmaceutical company, a FIPCO. Do you remember him talking about that goal? .

Yansura: Yes, very early on it was clear that Bob Swanson was very excited about the idea of having plants to make a protein. A lot of times he would have his picture taken with the big fermenter in the background. That fermenter barely worked, but he would always have his picture taken there. He would get really excited about that. So he didn't just want to make money; he wanted to produce something.

Brian Sheehan, First Vice President of Manufacturing

Hughes: Sometime in 1977 Brian Sheehan arrived as Vice President of Manufacturing. What was there to manufacture in 1977?

Yansura: Brian was a nice guy and I liked him, but we always made fun of him.

Hughes: Why?

Yansura: Here he was, VP of Manufacturing and we had nothing to manufacture. We'd be working our tails off in the lab to get insulin going, and he'd come in at nine o'clock in the morning and get out a push broom and clean off the loading dock. We just thought it was absurd. He had a rocky relationship with Bob Swanson. In fact, he had a poor relationship with all the scientists. We sat down a couple times together and talked about how we were going to make these proteins on a large scale. It was clear that he had no idea how we were going to do it. We didn't know for sure we could do it either, but at least we were going to try. He wasn't really into producing these things.

Eventually, he had an affair with Sharon Carlock, another person at Genentech that I liked personally. In a small company with twelve, fifteen people, it is hard to conceal this kind of thing. But Sharon Carlock was very catty. It wasn't really clear what her position was. We thought of her as a glorified secretary. She took care of the insurance, the paperwork, and did a good job with that. She was power hungry though, and looked at the scientists as people who worked for her. In particular, she and Dave Goeddel didn't quite hit it off. We took advantage of it, and while we were working hard we had something to complain or talk about. Eventually Bob Swanson fired both of them. I can't remember the exact cause, but it was clear that they were causing a division within the company. It was too small. We had to be really working together.

- Hughes: Why do you suppose that Swanson hired Sheehan so early in the game, before there was anything to manufacture?
- Yansura: I could never quite figure that out.
- Hughes: I wonder if it was a ploy to indicate to the investment community that Genentech was on its way to becoming a FIPCO.
- Yansura: That's true. You can't hire just anybody; you have to have somebody with an engineering degree to convince the investors.
- Hughes: Did Sheehan have a background that made him the right candidate for VP of Manufacturing?
- Yansura: I believe he did, but I can't recall exactly what his background was except that he was an engineer.

Anyway, the story goes on that he and Sharon got fired on the same day. Brian had gotten a lot of stock and it was worth in the tens of millions of dollars. Without doing a whole lot, he made himself into a multi-millionaire. The rumor is that he invested all of his money, with Sharon Carlock, in a new company which was going to make recombinant vaccines. As I mentioned, we were working on vaccines also for a while. But eventually the company went bankrupt, so he lost essentially everything.

More on Growth Hormone

The UCSF Growth Hormone Clones

- Hughes: Let's get back to human growth hormone. The main reason Seeburg was hired was to work on human growth hormone?
- Yansura: Swanson was very interested in hiring Peter Seeburg and Axel Ullrich. Dave Goeddel was already trying to clone human growth hormone. We had the synthetic DNA made for the

front end of the human growth hormone gene, and then it was attached to a promoter. He had all that in place. Dave Goeddel apparently eventually had clones with the rest of the gene. This has gone into the courts, and there are different versions of what happened. The version that I think is probably true is, Dave Goeddel had clones of human growth hormone. About the same time, Peter Seeburg and Axel Ullrich had a falling out with Howard Goodman, so they said, okay, we're going to go with Genentech. Peter Seeburg had brought some of the UCSF growth hormone clones here, and we were all worried about that.

Hughes: You were worried at the time?

Yansura: Well, yes. The lawyers were worried, and they told us that we should worry about that.

Hughes: Why?

Yansura: They didn't want to get into a lawsuit with UCSF, and they figured what Peter had done there belonged to UCSF and what we did here was ours.

Hughes: Was Tom Kiley one of the attorneys?

Yansura: I believe it was Tom Kiley who objected to Peter bringing this stuff with him. Normally, you tend to bring things with you. Most people don't worry about bringing research material that they had worked so hard on.

Protocol for Transferring Research Material

Hughes: Some of the problem, I suspect, was the novelty of the situation. There hadn't been many examples yet of molecular biologists moving with their cell lines or whatever to industry. Would there have been much precedent for that in 1978?

Yansura: There must have been a number of scientists that went directly out of universities into pharmaceutical labs.

Hughes: And they would have taken their material with them?

Yansura: Yes, I would think they would have taken it with them. But at the time there wasn't any financial incentive to do that; it was material that a scientist had worked on very hard, and he wanted to take it with him. Coming here, I'm sure I brought stuff from Marv Caruthers's lab, probably some lac operator DNA.

Hughes: And you didn't think twice about bringing it?

Yansura: I didn't think twice about it. Some of the other scientists brought stuff with them. We didn't think anything of it. Nowadays it would be different because everybody is trying to

get as much value as possible through patents on their stuff. Back then we didn't think about that.

Hughes: A university might argue that the cell line or whatever was created within the university so the university owns it. Particularly so if the research was supported by federal grants which come to the institution rather than the scientist. [pause] But I can also understand that the scientist who has spent effort in creating a cell line or a plasmid or whatever feeling that it is his or hers.

Yansura: Yes, it wasn't like he took it all; he just took a little sample.

Hughes: In your case, Caruthers wouldn't have cared that you had taken material with you?

Yansura: [hesitates] Probably not, because everybody shared things back then. Even Marv had samples from Khorana's lab. You just took a little bit of your stuff and that way you didn't have to write to your advisor and ask for this stuff, most of which would eventually get discarded. He would have to look in the freezer for the stuff that you wanted. Sometimes there was some kind of an agreement or at least a discussion that you could take this or that.

Hughes: There was the custom of acknowledging in a publication that a specimen had been given by another lab, right?

Yansura: Yes.

Early Concern Regarding the UCSF Clones

Hughes: It sounds as though there was an awareness that there were two sets of clones of different origins at Genentech. As soon as Peter arrived on the scene with his clones from UCSF, the attorneys raised your awareness that this situation was something to watch?

Yansura: Yes.

Hughes: Was any attempt made to keep the clones from different origins separate and distinct?

Yansura: Yes, they were kept separately. I recall that Peter Seeburg was told to take his stuff out of Genentech. He may have taken it home to his freezer, is my guess. I think that's what happened. The question in the courts was whether Dave Goeddel had his own clones for growth hormone. I feel pretty certain that Dave Goeddel had his own clones. Dave Goeddel is a very honest person.

Hughes: Was this situation a concern to scientists at Genentech? Or was this just another set of clones? I'm trying to get at how much awareness there was at the time of a potential problem here.

Yansura: Well, Tom Kiley pointed that out to us very clearly. It particularly was directed at Peter Seeburg. But Dave Goeddel knew that if he used Peter's stuff there could be problems. Unfortunately, the way the story goes is, Peter Seeburg did the last step for making the expression plasmid. He cut the human growth hormone gene out of a plasmid--supposedly Dave's clones--and stuck it in the plasmid that we had already constructed with the synthetic DNA for the front end--

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Hughes: You were saying?

Yansura: So Peter Seeburg took the human growth hormone gene and put it in the expression plasmid and that made the final step. To be honest, nobody really knows except Peter Seeburg which plasmid he cut this out of. So that step eventually ended up in the litigation with UC.

Hughes: Are you saying that Peter may have taken his clones to his home freezer and later brought them back to Genentech--or never taken them to his home freezer?

Yansura: Yes. I like Peter Seeburg; he's a nice guy. He seemed a little shady. That was his natural self.

Hughes: Meaning that you didn't quite know what he was doing?

Yansura: Yes, you didn't quite know what he was doing.

Hughes: But he must have been really alert to the situation, not only because of people like Tom Kiley at Genentech, but there was a furor when he took the clones from UCSF on New Year's Eve.

Yansura: Yes.

Hughes: There was a lot of controversy going on at the time--it was not any old set of clones.

Yansura: Well, yes, that's the way it went. [laughter]

Dave Goeddel's Involvement

Hughes: Then, I am told, the human growth hormone project didn't go particularly well under Peter Seeburg. Or is that Dave Goeddel's perception? In March or so of 1979 Dave became more involved in the project because he didn't think it was moving fast enough. Is that your perception?

Yansura: Yes. Dave was interested in really pushing it because he was concerned about the company. The company really needed another project to keep it going. Peter Seeburg was

not particularly interested in Genentech. He was more interested in whatever he was interested in at the time; he was interested in himself, so to speak. He continued to do this through his tenure at Genentech: He was only interested with doing his own research, and Genentech was just sort of there, paying him.

- Hughes: You mean, his research wasn't necessarily targeted on making a product for Genentech?
- Yansura: Right. He had his own academic interests in growth hormone, and they weren't exactly in making an expression plasmid and getting the protein expressed. And that's why Dave Goeddel took over.
- Hughes: Was Swanson aware of this situation?
- Yansura: I think Swanson was aware of what was going on.
- Hughes: Swanson, from the descriptions I've had of him, was very product oriented. He wanted the company to succeed. It's hard to imagine that he knowingly would let Seeburg indulge in his own scientific interests that weren't necessarily the company's.
- Yansura: Swanson may have thought, and correctly, that he could get Dave Goeddel to do it. He didn't want to irritate Peter Seeburg or push him out because Peter Seeburg had the growth hormone gene, and wherever Peter went the growth hormone gene went. That would be a bonus to our competitor.
- Hughes: Seeburg and Ullrich were courted by Swanson before Goeddel and you and Kleid came into the picture. They apparently were waffling, were not giving a yes or no answer.
- Yansura: Yes, they were academically inclined. To some extent they didn't really want to have to deal with a company. But they did; they were forced to.
- Hughes: Tell me what happened when Dave Goeddel stepped forcefully into the growth hormone project.
- Yansura: Well, even before Peter was here, Dave had the synthetic front end of the gene put together. His plan to express it was very concrete. I don't think Howard Goodman had a real plan to do that. So all Dave had to do was come up with the back part of the gene, and he eventually cloned it.

The Science

- Hughes: How much was the technology Genentech used for human growth hormone based on insulin and somatostatin?
- Yansura: Human growth hormone was different in that the protein was much bigger. The protein is 200 amino acids versus the insulin A and B chains are like fifty. So we couldn't make it

synthetically; it was too big. Nowadays, of course, we could do that easily. [animal clock chimes] So the plan was to use synthetic DNA to make the first part of the gene so that you could bring it in frame with a methionine for a start, and then use a restriction site that's naturally in the gene to connect it to the back part.

Hughes: What were you connecting?

Yansura: Well, the way the gene occurs naturally is it has a signal sequence at the start which codes for a leader peptide that comes before the mature part. The only thing that can be injected as a pharmaceutical is the mature part of the protein; that's the actual drug that we wanted to make.

Hughes: What do you mean by mature part?

Yansura: Normally, proteins that are secreted originally have a signal sequence on the front, which is usually twenty to thirty amino acids. It helps to get the protein through the membrane. Then it gets cleaved off. What remains is considered the mature part of the protein.

Hughes: That's what you want.

Yansura: That's what you want. You don't want the signal sequence. The signal sequence is there just to move things around. In fact, it's really a problem if you come up with a protein with the signal sequence still attached; you can't really do anything with it. You can't inject it as a pharmaceutical because it will be immunogenic. You need it cleaved off. We had already come up with a way to do that, and Howard Goodman obviously hadn't a plan to do that.

The Goodman Group's Problem

Hughes: My knowledge is shaky, but the Goodman group did not have the mature molecule, as you've described it. There were sequences that they didn't want.

Yansura: Yes, and they didn't have a way to get them off. Whereas with human insulin and somatostatin, we made fusion proteins, but we had a chemical way to cleave off the leader protein. The Goodman group had no way to cleave off their signal sequence. In a lot of ways they relied on the same strategy as Wally Gilbert to make insulin. Wally Gilbert thought that if you had the whole cDNA for human insulin, you would initially have a signal sequence out front, but that would get cleaved off later by the bacteria when the protein got secreted. Of course the bacteria didn't want to secrete it, so they left it with the signal sequence still on it. Howard Goodman's group ran into the same problem. They eventually made something with some fusion on the front, which had part of the signal sequence or something--I can't remember--but it wasn't useable. We were interested in making something useable that you could turn into a drug, inject into humans, take to clinical trials.

Hughes: Is it possible that Goodman's group was interested in more basic science questions? Was cloning at an early enough stage that it was of interest to see what protein one would get through cDNA methodology and compare that to the genetic code?

Yansura: I think the sequence and the genetic code were pretty much a certainty by then. What wasn't clear was the rules for expressing proteins. There essentially weren't any rules, except everybody knew you had to have a promoter upstream of the gene to make mRNA. That was about the only rule available. Most genes of interest, and essentially all of the genes of interest very early on and even since then that have been turned into recombinant proteins, have all been secreted proteins. They all occur naturally with signal sequences on their N-terminus. The academic labs--Wally Gilbert's and Howard Goodman's--were perhaps intrigued that they could get it cleaved off easily in bacteria. It would have been very impressive and elegant to do that at that early stage.

We thought differently here at Genentech. We thought more like engineers, in some sense, in that we said, "We don't know if we can get that signal sequence cleaved off." Nowadays we can do that but back then we couldn't. So the plan was to make [the protein] without that signal sequence. We made our synthetic DNA, so we totally eliminated the signal sequence. We started with a methionine and went right into the mature gene. That way we didn't have to worry about what would happen: Will the bacteria process that signal sequence or not? We didn't have to deal with that at all; we simply had to make the protein.

Hughes: The situation reflects different goals, doesn't it? Genentech wanted a product that could be commercialized; the academic labs might have found it very nice to produce growth hormone, but that wasn't all that they were after; they had basic science interests as well.

Yansura: That's true, yes.

More on the Science

Hughes: There were several new aspects about what you were trying to do with human growth hormone. One, as you alluded, was that the size of the molecule was four times that of insulin. There were also the synthetic and non-synthetic aspects to the work, and probably more I don't know about. What did you have to learn to produce growth hormone that maybe you hadn't had to do when Genentech was working on insulin or somatostatin?

Yansura: For one thing, insulin and somatostatin were made as huge fusions to beta-galactosidase. Beta-galactosidase has been well studied, and it's a huge protein. What everyone thought they were doing was tricking the bacteria: Adding a few extra amino acids on the back and *E. coli* would make this protein and not care. With human growth hormone, we were going to make the mature protein directly in the bacteria--no fusion. It's big. Personally, I didn't know how easy or difficult it was going to be to make that protein. It turned out that as soon as Dave Goeddel got the clones for the final expression construct, he immediately tried to induce the promoter and got fairly high levels of expression. To me it was

surprising. I remember Herb Heyneker was there also very excited, and it was pretty amazing to be able to see that.

Hughes: Were you looking at the gels? Is that how you knew that growth hormone had been produced?

Yansura: Yes, you could see it on an SDS gel. There was a lot of excitement because we didn't know how difficult it was going to be. In retrospect, there was some luck involved. If you did that with ten different genes in that same time frame you'd probably have expressed only a third of them.

Hughes: What part of this work were you doing?

Yansura: After working on insulin, I sequenced the human growth hormone gene that Dave Goeddel cloned to make sure it was correct and so forth.

Protein Folding

Hughes: Any other experimental problems before you actually produced human growth hormone?

Yansura: Yes. We knew it was being made, and I believe we knew that at least some of it was soluble and active. I can't remember exactly how we knew that, but we knew that early on. But as we tried to increase the expression levels to make a product out of it, and we started producing it in the fermenters, we noticed that essentially all the protein was insoluble. It was misfolded. The protein had to be refolded.

Hughes: How did you do that?

Yansura: I didn't do that. It turns out that it refolds fairly easily. You have to solublize the protein with guanadine or urea, and then you dilute out these agents, and the protein just folds nicely.

Hughes: Did you know that in advance?

Yansura: No. So again we were lucky.

Hughes: Was that a trial-and-error approach?

Yansura: Yes, that's how you normally do the refolding, even today. But we were lucky because refolding technology back then was not very advanced.

Hughes: Were there later products where folding was a problem?

Yansura: Yes, many of them.

Hughes: What was the next one, do you recall?

Yansura: [pause]

Hughes: One of the interferons maybe?

Yansura: I think some of the interferons folded right away. Some misfolded but could be refolded fairly easily. tPA was thought to be impossible to refold. That's not the case. There is a company that sells a refolded tPA made in bacteria and competes with Genentech.

We were lucky in the early days that these were proteins that behaved nicely. Looking back, you realize there was luck in the expression; there was luck in the refolding.

Growth Hormone With and Without Methionine

Hughes: What is the significance of human growth hormone with and without methionine?

Yansura: The first growth hormone we made in the cytoplasm without a signal sequence. I believe the first mature amino acid is phenylalanine. You can't start protein translation in any organism with phenylalanine; it always starts with methionine, and that's universal. So we had to add a methionine to start the translation. Then it translates phenylalanine and so forth of mature human growth hormone. The protein we made has one extra amino acid on it, and that's a methionine at the very beginning. That was somewhat a concern, not a major concern, at the time. One possibility was that it would be antigenic in humans. Again we lucked out. It wasn't antigenic, and in fact a number of proteins are made with an extra met, and none of them apparently have any problems. That was our first version of human growth hormone.

Maybe about 1986 or so we developed technology to secrete proteins in *E. coli*. We would start out with the signal sequence version of human growth hormone and replace the signal sequence with a bacterial signal sequence, and then stick it back in *E. coli*, and *E. coli* would secrete it, and it would process and remove that signal sequence.

Hughes: Did growth hormone with methionine impact the FDA approval process?

Yansura: There was some impact. When we started clinical trials, there were some cases where there were antibodies generated against our human growth hormone. It wasn't terribly high levels of antibody, so we could keep the program going and get approval. But it was something we had to follow. I'm not sure if the FDA asked us to do that or not. I think they probably did. So we followed it for a number of years. The original theory was that the extra methionine was antigenic. But eventually the research proved that the methionine had nothing to do with it. We were making growth hormone with and without met for a while, and they both gave rise to low levels of antibodies against it in some patients. I'm not sure if we still make both versions.

Partnership with KabiVitrum

- Hughes: Can you tell me something about the relationship between Genentech and Kabi, your industrial partner in the growth hormone business?
- Yansura: Yes. Kabi had the skills to sell growth hormone in Europe. So we very early had agreements with them. We would develop the technology, transfer it to them, and they would sell it in Europe, and we would sell it in the US.
- Hughes: Was the arrangement similar to the one on insulin with Lilly?
- Yansura: It was different in that with insulin we gave everything to Lilly, and they gave us some royalties. We wanted to make growth hormone here and sell it in the U.S., which was a big enough area to cover for us. We couldn't handle international sales at this time in our development. I always thought Kabi was a good partner to work with.
- Hughes: Did Genentech work directly with Kabi scientists, or did it mainly involve sending cell lines or plasmids back and forth?
- Yansura: A lot of it was sending things back and forth.
- Hughes: They didn't come here and you didn't go there?
- Yansura: I didn't go there myself. Some people I worked with went there eventually. And we had conference calls.

Expansion of the Growth Hormone Market

- Hughes: At the same time, UCSF had a contract with Lilly for growth hormone.
- Yansura: We were the only source of growth hormone until Lilly came up with their own version which they got through interacting with UCSF.
- Hughes: When might that have been?
- Yansura: It must have been a couple years later. Initially, we were sharing the market with cadaver-generated human growth hormone, but then some kids taking it came down with one of the prion diseases such as CJD [Creutzfeldt-Jakob disease]. That was the immediate end to cadaver growth hormone. It was sad, certainly, but for us it was good because now we had the whole market; there was no other source.
- Hughes: You continue to have the whole market today?

- Yansura: We share it now with Lilly. There are a couple of other players worldwide, Serono and so forth. We have the major share of it, in the U.S. at least.
- Hughes: Kabi is still a player.
- Yansura: Yes, Kabi is still making it.
- Hughes: So the benchmarks that had been so prominent in the contract with Lilly were not a feature of the relationship with Kabi?
- Yansura: There were benchmarks, but I don't remember them being important at all. I think it's because we were driving growth hormone towards the market for ourselves, and then whatever we had developed we gave to Kabi. With insulin, we had to develop and give it to Lilly because we desperately needed the benchmark money.

Clinical Trials Involving Genentech Employees

- Hughes: Probably in 1981, twelve or so Genentech employees were injected with human growth hormone. Do you remember that, and were you maybe one of them?
- Yansura: I was not one of them. I remember they offered some money to do that. It was \$100 or \$500, something like that. I thought about it, but I decided I just didn't want to do it. The first reaction was, they all got very sore at the injection site, and they had flu-like symptoms. Apparently it was due to an endotoxin .
- Hughes: Does that mean a contamination?
- Yansura: *E. coli* is just loaded with endotoxin; it's part of the cell membrane and so forth. Your body is very sensitive to endotoxin because it thinks that *E. coli* is breaking through your intestine walls, so it massively attacks it. They cleaned it up a little after that, and it seemed to be fine.
- Hughes: Is using Genentech employees as guinea pigs repeated in Genentech history?
- Yansura: I think that was the only time. They have offered money for blood, but I think that was the only time people got injected.
- Hughes: What about the scale-up of human growth hormone? Was that something that Kabi handled, or was Genentech also involved?
- Yansura: We did our own scale-up here. That was the driving force. Kabi had to do it in Sweden on their own. Well, not totally on their own. We would develop it here, and when we had something, we would transfer it to them. The most important thing for us was to get it marketed ourselves.

Growth Hormone As Indicator of Genentech's Success As a Business

- Hughes: Insulin, we know, made a stir in the press. Was there something about the success with human growth hormone that moved the company or moved the industry a step further?
- Yansura: Growth hormone didn't have the big splash that insulin had, and that's because everybody knows what insulin is; everybody doesn't know what human growth hormone is. So it didn't have that impact in the press. But it did have the impact of being the first protein that a biotech company took to the market by itself.
- Hughes: Was the investment community impressed with that accomplishment?
- Yansura: Yes, I think it was way more impressive than the insulin was. When we first got insulin expressed and had the big press conference, it wasn't clear that we could make enough of it to turn it into a product that you could sell. When growth hormone came out, and we showed that we could make it in fairly large quantities, enough to supply the market, that was much more impressive to the financial community.
- Hughes: Dennis Kleid told me that with the human growth hormone success he could rest assured that the company had a chance of making it. It had achieved an important goal.
- Yansura: Yes, I think that's right. The feeling was that we couldn't make it on the royalties we were going to get from insulin. In fact, our original growth hormone market wasn't that huge. It was much smaller than it is now. Then when the cadaver growth hormone ceased to exist, the market just seemed to explode, and we were all of a sudden making hundreds of millions. I think the original estimate was \$15 million. Someone was quoted as saying that growth hormone would pay for the toilet paper at Genentech for one year. In other words, it wasn't that much. But it turned out to be much bigger than we thought.

Criticism of Business Practices

- Hughes: Genentech was later criticized for alleged over-marketing of growth hormone. Genentech pushed it for treatment for conditions in addition to small stature. Is that one reason for the under-estimation of income from growth hormone?
- Yansura: That's a good question. Before there was recombinant growth hormone, there was always an under supply--there wasn't enough growth hormone to meet demands. That in itself kept the demands somewhat smaller. Now, all of a sudden, you had essentially unlimited amounts of growth hormone available. Then people started to think, "Sure, why not?" It's a different story than if you have to beg for some from a short supply. I think that was the main reason that the market took off. Certainly there was some over-selling, overzealousness.

- Hughes: There was a kickback scandal, probably in the late 1980s. Do you remember any of that history, and how it felt here at Genentech to have that happening?
- Yansura: Genentech started off as a renaissance company, and then it went into the Dark Ages. This was when we hired Kirk Raab to be the CEO. Bob Swanson was very good in the early days. He knew how to deal with the venture capital markets and so forth. But the company got to a point where we had to behave to some extent like a normal pharmaceutical company. So Bob Swanson hired Kirk Raab. It was during that period that we got into this problem with kickbacks and so forth. Kirk Raab was definitely more of a marketing person, so he pushed the marketing sector to over-perform. Most of the people at Genentech felt badly about it, but what can you do?

Limited Clinical Partnerships

- Hughes: In January of 1983 Genentech founded a clinical partnership which was a funding mechanism for clinical trials of human growth hormone. Another partnership was founded when tPA came along. It was a new financial strategy, as I understand it.
- Yansura: You're talking about the limited partnerships. Those went out of favor during the Reagan years.
- Hughes: It was a tax shelter?
- Yansura: Yes.
- Hughes: Did Genentech employees invest in them?
- Yansura: Yes, some of them did. I think some of them came out okay and some didn't. I thought it was way too risky, although I did invest in other limited partnerships and lost a lot of money.
- Hughes: Was that a unique strategy for funding clinical trials?
- Yansura: I believe that was the first one. Remember, the large pharmaceutical houses pay for their own clinical trials, and they're not going to have a limited partnership for some drug. It's something that only some struggling little company will do. I believe Swanson was the first one to do that. He got a lot of credit for doing it.¹

¹See the oral history in this series with Fred Middleton.

Orphan Drug Status

Hughes: Human growth hormone had orphan drug status.

Yansura: That's correct.

Hughes: What difference did that make?

Yansura: There's a law that says you can get orphan status for a drug if--I can't remember what the exact number of cases is--the patient population is below a certain threshold. Once you get the status, it prevents another company from coming in with a similar product for so many years. So we had gotten orphan status for growth hormone. It kept competitors away for a while.

Hughes: Was that the only drug for which Genentech got orphan drug status?

Yansura: Yes.

UC v. Genentech, 1999: Rehearsing an Old Experiment

Hughes: You've mentioned in passing the recent UC-Genentech case involving human growth hormone. Did you testify?

Yansura: I did not testify at the trial, although I had a number of days of depositions. It was interesting to hear about this from the other side. Peter Seeburg put the gene into the final expression plasmid. The question is, did he put his gene in or did he put Dave Goeddel's gene in? They figured out from Peter's notebook how much time he had to do that. It was something like eight hours. The opposition, UC, said that he couldn't physically do it in eight hours. Dennis Henner was VP of Research at the time. He asked me if I could practice doing this so we could make it under eight hours. I practiced cutting the plasmids, running the gels, ligating and so forth, and making a fragment in eight hours. We were able to do it, but it was at that point that I realized I was working awfully fast to do this and that was not Peter Seeburg's personality, doing that kind of work. [laughs] Then I got to thinking that maybe he did put UC's gene in there. His own growth hormone clone was available before Dave had gotten his, and he would have had plenty of time to cut out his gene beforehand. Only he really knows.

Hughes: Did you have to say that?

Yansura: I did not have to say that. This was something that came to me later. We actually had a three-way trial on growth hormone with UC and Lilly. It was because Lilly was using our promoter and our technology to make their human growth hormone, and UC thought we were using their gene, so we had this three-way litigation. So I spent a lot of time doing depositions. It wasn't until later when I tried to repeat Peter Seeburg's work that I thought

that maybe he did use his own fragment. In fact, I think he probably had his gene fragment sitting there, just waiting to be used.

Hughes: Sitting there at Genentech, you mean?

Yansura: Yes. [tape interruption]

David Martin As Vice President of Research, 1983-1990

Hughes: In 1983, David Martin became Genentech's first official Vice President of Research.

Yansura: It's a question: Why did it take so long for us to have a VP of Research? In the very early days, probably the first year or two, it didn't matter in that everybody was highly motivated, and we had very discrete projects: insulin, growth hormone, foot-and-mouth, the interferons, etcetera. So things moved fairly smoothly.

We eventually got to a size where we couldn't deal with all these scientists. The question was, who would be VP of Research? Dave Goeddel was an obvious candidate. He was well respected, and he had put a lot of energy into making Genentech what it was. Dave Goeddel is a hard person, and I think that is perhaps why it didn't go to him. Genentech needed someone able to deal with a lot of different scientists. Eventually, they found Dave Martin. He is a very smart guy. He has a nice personality; you can talk with him. He does tend to be a micro-manager and likes to control everything. It didn't work out very well. Eventually, all the scientists realized he couldn't do it and that it was time to find someone else.

Hughes: What was work like before and after Martin arrived. How did his presence affect your productivity and attitude?

Yansura: It was much nicer and people were more motivated in the early days with no research director. There were projects and everybody knew what they were doing and everybody talked and there wasn't a lot of conflict. I can't blame it on Dave Martin. At the time he came, research was growing and you needed somebody to be on top of everything. It may have been him; it may have been the times, but things were much harder when he came.

Hughes: Whose decision was it to create the position?

Yansura: I think Swanson always had that in mind. I don't know what was going on behind the scenes. My guess is Dave Goeddel was the obvious choice, and yet I could see why some scientists would resist. The scientists have to say, I respect this guy and can have him as my leader. Dave Goeddel was respected, but he has a harsh personality at times, and some scientists wouldn't go along with him as VP.

Hughes: As I remember, David Martin came directly from UCSF.

Yansura: Yes, I believe he did.

Hughes: Were you aware of a culture clash? An academic environment is different from a corporate environment.

Yansura: Yes, I think there always is somewhat of a clash when you get someone from the outside coming in because Genentech is not an academic culture. Compared to the big pharmaceutical companies, yes it is, but it's real different here than it is at UCSF. I think Dave Martin wanted to fit in, and he molded himself to the Genentech culture. So in some ways he fit in very well. He was the first one to bring in the espresso machines. I think people liked him at first. It wasn't that people disliked him, but eventually he became too much of a micro-manager. He always thought he had the right ideas, and it should go his way. He always had his hands in there, and that's what drove everybody crazy.

Hughes: What about the different goals of science in academia and in a corporate setting? Traditionally--and I know there are a lot of variations--science in academia explores basic questions, and the goal in industry is products. Does a Vice President of Research whose career up until then has had an academic bent tend to present problems for the scientists and hence for the company he's moving into?

Yansura: Yes, it can. In some ways we're in the same boat now with Richard Scheller coming from academia. I think it can work. Herb Boyer was in academia, and yet he fit right into the Genentech culture; he was product oriented.

One of the problems Dave Martin had was he never could come up with new products to go into the pipeline. As VP of Research that's probably your main goal, and you have to massage the research group to pull that off. He couldn't do that, and he ended up trying to micromanage everything to try to do that, and that didn't work either.

Hughes: How do you massage a group to move it along to a product?

Yansura: That's a good question. If I knew, I'd be in Richard Scheller's position. When Genentech first started everybody realized that our next paycheck depended on us making something to go in the pipeline and we were very focused. Even before human growth hormone got approved, there was a change in research culture. A lot had to do with Peter Seeburg and Axel Ullrich coming. They were more interested in doing basic research. That was very different than Dave Goeddel's original focus which was on producing products, getting them into the pipeline. Dave Goeddel started to shift over also after a while. All of research started to shift from really focused projects to make products, to doing basic research. That's when Dave Martin was in charge of research. I don't want to blame it all on him--that's the way research was going. But he was the leader. Now, things are very focused, almost like they were initially, on filling up the pipeline with products.

Contributions

- Hughes: Well, it's time that we wound up these discussions. What do you think of as your greatest contribution?
- Yansura: Oh my. That's a hard question. Scientists don't like to talk about that.
- Hughes: All right, I'll restate the question: What have you done for the company that you're most proud of?
- Yansura: I like to think of my work that has been a part of pushing some pharmaceutical to the market, for example, growth hormone and insulin. My work on the expression of those proteins has been incorporated into the production process. That gives me a connection with these two proteins.
- Hughes: [animal clock chimes] Well, the clock is telling us that you need to go. I thank you very much.

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PUBLICATIONS

Szeto, W., Jiang, W., Tice, D.A., Rubinfeld, B., Hollingshead, P.G., Fong, S.E., Dugger, D.L., Pham, T., Yansura, D.G., Wong, T.A., Grimaldi, C., Corpuz, R.T., Singh, J.S., Frantz, G.E., Devaux, B., Crowley, C.E., Schwall, R.H., Eberhard, D.A., Rastelli, L., Polakis, P., Pennica, D. Overexpression of the retinoic acid-responsive gene *Stra6* in human cancers and its synergistic induction by Wnt-1 and retinoic acid. *Cancer Res.* 61:4197-4205 (2001).

Pan, G., Risser, P., Mao, W., Baldwin, D.T., Zhong, A.W., Filvaroff, E., Yansura, D., Lewis, L., Eigenbrot, C., Henzel, W.J., Vandlen, R. IL-1H, an interleukin 1-related protein that binds IL-18 receptor/IL-1Rrp. *Cytokine* 13:1-7 (2001).

Lee, J., Ho, W.S., Maruoka, M., Corpuz, R.T., Baldwin, D.T., Foster, J.S., Goddard, A.D., Yansura, D.G., Vandlen, R.L., Wood, W.I., Gurney, A.L. IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1. *J. Biol. Chem.*, 276(2):1660-1664 (2001).

Bass, S.H., Yansura, D.G. Application of the *E. coli* trp promoter. *Mol. Biotechnol.* 16:253-260 (2000).

Baly, D.L., Horuk, R., Yansura, D.G., Simmons, L.C., Fairbrother, W.J., Kotts, C., Wirth, C.M., Gillece-Castro, B.L., Hesselgesser, J., Allison, D.E. A His19 to Ala mutant of melanoma growth-stimulating activity is a partial antagonist of the CXCR2 receptor. *J. Immunol.*, 161(9):4944-9 (1998).

Horuk, R., Reilly, D., Yansura, D.G. Expression, purification, and characterization of *Escherichia coli*-derived recombinant human melanoma growth stimulating activity. *Methods in Enzymology*, 287:3-12 (1997).

Yansura, D.G., Bass, S.H. Application of the *E. coli* trp promoter. *Methods in Molecular Biology*, 62:44-62 (1997).

Simmons, L.C., Yansura, D.G. Translational level is a critical factor for the secretion of heterologous proteins in *Escherichia coli*. *Nature Biotech.*, 14(5):629-34 (1996).

Mehlhorn, I., Groth, D., Stockel, J., Moffat, B., Reilly, D., Yansura, D., Willett, W.S., Baldwin, M., Fletterick, R., Cohen, F.E., Vandlen, R., Henner, D., Prusiner, S.B. High-level expression and characterization of a purified 142-residue polypeptide of the prion protein. *Biochem.*, 35(17):5528-37 (1996).

Hesselgesser, J., Chitnis, C.E., Miller, L.H., Yansura, D.G., Simmons, L.C., Fairbrother, W.J., Kotts, C., Wirth, C., Gillece-Castro, B.L., Horuk, R. A mutant of melanoma growth stimulating activity does not activate neutrophils but blocks erythrocyte invasion by malaria. *J. Biol. Chem.*, 270(19):11472-6 (1995).

Seymour, J.L., Lindquist, R.N., Dennis, M.S., Moffat, B., Yansura, D., Reilly, D., Wessinger, M.E., Lazarus, R.A. Ecotin is a potent anticoagulant and reversible tight-binding inhibitor of factor Xa. *Biochem.*, 33(13):3949-58 (1994).

Horuk, R., Yansura, D.G., Reilly, D., Spencer, S., Bourell, J., Henzel, W., Rice, G., Unemori, E. Purification, receptor binding analysis, and biological characterization of human melanoma growth stimulating activity (MGSA). *J. Biol. Chem.*, 268: 541-546 (1993).

Yansura, D.G., Simmons, L.C. Nucleotide sequence selection for increased expression of heterologous genes in *Escherichia coli*. *In: Methods: A companion to Methods in Enzymology*, 4: 151-158 (1992).

Holmes, W.E., Sliwkowski, M.X., Akita, R.W., Henzel, W.J., Lee, J., Park, J.W., Yansura, D., Abadi, N., Raab, H., Lewis, G.D., Shepard, H.M., Kuang, W., Wood, W.I., Goeddel, D.V., Vandlen, R.L. Identification of heregulin, a specific activator of p185erb2. *Science* 256: 1205-1210 (1992).

Yansura, D.G. Expression as a trpE fusion. *Methods in Enzymology*, 185:161-6 (1990).

Yansura, D.G., Henner, D.J. Use of *Escherichia coli* trp promoter for direct expression of proteins. *Methods in Enzymology*, 185:54-60 (1990).

Paborsky, L.R., Tate, K.M., Harris, R.J., Yansura, D.G., Band, L., McCray, G., Gorman, C.M., O'Brien, D.P., Chang, J.Y., Swartz, J.R., Fung, V.P., Thomas, J.N., Vehar, G.A. Purification of recombinant human tissue factor. *Biochem.*, 28:8072-77 (1989).

Robertson, B.H., Grubman, M.J., Weddell, G.N., Moore, D.M., Welsh, J.D., Fisher, T., Dowbenko, D.J., Yansura, D.G., Small, B., Kleid, D.G. Nucleotide and amino acid sequence coding for polypeptides of foot-and-mouth disease virus type A12. *J. Virology*, 54(3):651-60 (1985).

Weddell, G.N., Yansura, D.G., Dowbenko, D.J., Hoatlin, M.E., Grubman, M.J., Moore, D.M., Kleid, D.G. Sequence variation in the gene for the immunogenic capsid protein VP1 of foot-and-mouth disease virus A. *Proc. Natl. Acad. Sci. USA*, 82(9):2618-22 (1985).

McKercher, P.D., Moore, D.M., Morgan, D.O., Robertson, B.H., Callis, J.J., Kleid, D.G., Shire, S.J., Yansura, D.G., Dowbenko, D., Small, B. Dose-Response evaluation of a genetically engineered foot-and-mouth disease virus polypeptide immunogen in cattle. *Am. J. Vet. Res.*, 46(3):587-590 (1985).

Band, L., Yansura, D.G., Henner, D.J. Construction of a vector for cloning promoters in *Bacillus subtilis*. *Gene*, 26(2-3) (1983).

Yansura, D.G., Henner, D.J. Use of *Escherichia coli* lac repressor and operator to control gene expression in *Bacillus subtilis*. *Proc. Natl. Acad. Sci.*, 81(2):614-23 (1984).

Robertson, B.H., Morgan, D.O., Moore, D.M., Grubman, M.J., Card, J., Fischer, T., Weddell, G., Dowbenko, D., Yansura, D. Identification of amino acid and nucleotide sequence of the foot-and-mouth disease virus RNA polymerase, *Virology*, 126(2):614-23 (1983).

Yansura, D.G., Dowbenko, D., Weddell, G.N., Hoatlin, M.E., Shire, S.J., Bock, L.A., Patzer, E.J., Kleid, D.G. Biosynthetic Vaccine for foot-and-mouth disease. *Advances in Gene Technology: Molecular Genetics of Plants and Animals*, 479-93 (1983).

Liu, C.C., Yansura, D.G., Levinson, A.D. Direct expression of hepatitis B surface antigen in monkey cells from an SV40 vector. *DNA*, 1(3):213-21 (1982).

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. Expression in *Escherichia coli* of a chemically synthesized gene for a "mini-c": analog of human proinsulin. *Gene*, 16(13):63-71 (1981).

Kleid, D.G., Yansura, D., Small, B., Dowbenko, D., Moore, D.M., Grubman, M.J., McKercher, P.D., Morgan, D.O., Robertson, B.H., Bachrach, H.L. Cloned viral protein vaccine for foot-and-mouth disease: responses in cattle and swine. *Science*, 214(4525):1125-9 (1981).

Riggs, A.D., Itakura, K., Crea, R., Hirose, T., Kraszewski, A., Goeddel, D., Kleid, D., Yansura, D.G., Bolivar, F., Heyneker, H.L. Synthesis, cloning and expression of hormone genes in *Escherichia coli*. *Recent Prog. Horm. Res.*, 36:261-76 (1980).

Yansura, D.G., Goeddel, D.V., Kandu, A., Caruthers, M.H. Studies on gene control regions IX. The effect of hypoxanthine-substituted lac operators on the lac operator--lac repressor interaction. *J. Mol. Biol.*, 133(1):117-35 (1979).

Goeddel, D.V., Heyneker, H.L., Hozumi, T., Arentzen, R., Itakura, K., Yansura, D.G., Ross, M.J., Miozzari, G., Crea, R., Seeburg, P.H. Direct expression in *Escherichia coli* of a DNA sequence coding for human growth hormone. *Nature*, 281(5732):544-8 (1979).

Goeddel, D.V., Kleid, D.G., Bolivar, F., Heyneker, H.L., Yansura, D.G., Crea, R., Hirose, T., Kraszewski, A., Itakura, K., Riggs, A.D. Expression in *Escherichia coli* of chemically synthesized genes for human insulin. *Proc. Natl. Acad. Sci.*, 76(1):106-10 (1979).

Goeddel, D.V., Yansura, D.G., Caruthers, M.H. How lac repressor recognizes lac operator. *Proc. Natl. Acad. Sci.*, 75(8):3578-82 (1978).

Goeddel, D.V., Yansura, D.G., Winston, C., Caruthers, M.H. Studies on gene control regions. VII. Effect of 5-bromuracil-substituted lac operators on the lac operator-lac repressor interaction. *J. Mol. Biol.*, 123(4):66-87 (1978).

Sadler, J.R., Betz, J.L., Tecklenburg, M., Goeddel, D.V., Yansura, D.G., Caruthers, M.H. Cloning of chemically synthesized lactose operators. II. EcoRI-linkered operators. *Gene*, 3(3):211-32 (1978).

Sadler, J.R., Tecklenburg, M., Betz, J.L., Goeddel, D.V., Yansura, D.G., Caruthers, M.H. Cloning of chemically synthesized lactose operators. *Gene*, 1(5-6):305-21 (1977).

Goeddel, D.V., Yansura, D.G., Caruthers, M.H. Studies on gene control regions. VI. The 5-methyl of thymine, a lac repressor recognition site. *Nucl. Acids Res.*, 4(9):3039-54 (1977).

Goeddel, D.V., Yansura, D.G., Caruthers, M.H. Blinding of synthetic lactose operator DNAs to lactose repressors. *Proc. Natl. Acad. Sci.*, 74(8):3292-6 (1977).

Yansura, D.G., Goeddel, D.V., Cribbs, D.L., Caruthers, M.H. Studies of gene control regions. III. Binding of synthetic and modified synthetic lac operator DNAs to lactose repressor. *Nucl. Acids Res.*, 4(3):723-37 (1977).

Yansura, D.G., Goeddel, D.V., Caruthers, M.H. Studies on gene control regions 2. Enzymatic joining of chemically synthesized lactose operator deoxyribonucleic acid segments. *Biochem.*, 16(9): 1772-80 (1977).

Goeddel, D.V., Yansura, D.G., Caruthers, M.H. Studies on gene control regions 1. Chemical synthesis of lactose operator deoxyribonucleic acid segments. *Biochem.*, 16(9):1765-72 (1977).

Goeddel, D.V., Yansura, D.G., Kawashima, E., Gadek, T., Caruthers, M.H. Synthesis of lac and lambda operator DNA sequences. *ICN-UCLA Symp. Mol. Cell. Biol. 5 Mol. Mech. Control Gene Expression* 159-64 (1976).

PATENTS

Methods and Compositions for Secretion of Heterologous Polypeptides. Simmons, L.C., Yansura, D.G. U.S. Patent Number 6,242,177 June 5, 2001.

Bacterial Polypeptide Expression Employing Tryptophan Promoter-Operator. Kleid, D.G., Yansura, D.G., Heynecker, H.L., Miozzari, G.F. U.S. Patent Number 5,888,808 March 30, 1999.

Methods and Compositions for Secretion of Heterologous Proteins. Simmons, L.C., Yansura, D.G. U.S. Patent Number 5,840,523 November 24, 1998.

Methods and Compositions for Secretion of Heterologous Polypeptides. Simmons, L.C., Yansura, D.G. U.S. Patent Number 5,747,662 May 5, 1998.

Process for Producing Relaxin. Breece, T., Hayenga, K., Rinderknecht, E., Vandlen, R., Yansura, D.G. U.S. Patent Number 5,759,807 June 2, 1998.

Processes and Compositions for the Isolation of Human Relaxin. Henner, D.J., Vandlen, R.L., Wilkins, J.A., Yansura, D.G. U.S. Patent Number 5,464,756 November 7, 1995.

Portable Inducible Control System. Henner, D.J., Yansura, D.G. U.S. Patent Number 4,912,046 March 27, 1990.

Preparation of Hepatitis B Surface Antigen in Yeast. Hitzeman, R.A., Levinson, A.D., Yansura, D.G., U.S. Patent Number 4,803,164 February 7, 1989.

Preparation of Polypeptides in Vertebrate Cell Culture. Levinson, A.D., Liu, C.C., Yansura, D.G., U.S. Patent Number 4,741,901 May 3, 1988.

Method of Altering Double-stranded DNA. Kleid, D.G., Yansura, D.G., Heynecker, H.L., Miozzari, G.F. U.S. Patent Number 4,663,283 May 5, 1987.

GENENTECH, INC.
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SOUTH SAN FRANCISCO, CALIFORNIA 94080
(415) 952-0123

December 20, 1978

Ed Smithwick
ELI LILLY AND COMPANY
Indianapolis, Indiana 46206

Dear Ed:

I am writing to confirm our conversation of 18 December 1978 concerning our tentative purification schemes for human insulin A and B chain recoveries.

1) Cell Lysis. We have carried out successful chemical lysis of RV308/pIB1 and RV308/pIA1 in 500 g (wet cell weight) batches using the following protocol:

- 500 g frozen cells suspended in 2000 ml of 10% sucrose. 0.2 M NaCl, 0.1 M Tris pH 7.9. Lysozyme added to 0.1 mg/ml. Stir 1 hour @ 4°C.
- Add 1 µg/ml RNAase and DNAase to the mixture followed by 100 ml of 20% Triton X-100. A sharp increase in viscosity followed by a slow drop in same was noted.
- The debris and the β-gal was centrifuged out.

2) A chain purification. Overall yield after CNBr of 70%.

- pH5 supernatant from S-SO₃ lyophilized and washed in 1 M HOAc.
- Pellet from above step dissolved in 0.01 M NH₄HCO₃ and loaded on AE cellulose column. Gradient run to .2 M NH₄HCO₃. A peak is at about .08 M NH₄HCO₃.
- This material lyophilized and loaded on G-50 fine Sephadex column in 0.05 M NH₄HCO₃. The peak is about half way between salt and void volumes. Greater than 90% pure by HPLC.

Ed Smithwick
 December 20, 1978
 Page 2

- HPLC conditions for A chain - SO_3 : 0.05 M NH_4OAc , pH 7.2
 6 to 20% CH_3CN gradient over 15' on ODS (RP-18) column. Peak
 is at ~ 10% CH_3CN .

3) B chain purification. Overall yield after CNBr 50% - 70%.

- pH5 pellet from S- SO_3 suspended in 6 M urea, 1 M HOAc and
 centrifuged.
- Supernatant loaded on SP Sephadex C-25 column and a gradient
 run to 0.25 M NaCl. Peak is at ~ 0.18 M NaCl.
- Dialyzed to 0.01 M Tris pH 8.2, 6 M urea after adjusting
 pH with Tris base to 8.2.
- Loaded on DEAE Sephacel and a gradient run to 0.1 M NaCl.
 Peak is about at 0.05 M NaCl. Concentrate. (~ 40% pure)
- Load on G-75 Fine Sephadex in 6 M urea, Tris pH 8.2. (This
 step has not yet been done, but most of the impurities at
 this stage are of higher molecular weight).
- HPLC conditions for B- SO_3 : 0.05 M NH_4OAc , pH 7.2
 30% - 45% CH_3CN gradient over 20' or
 40% - 60% Methanol gradient over 20'

I hope this information is of help if you wish to repeat our isolations.

We hope to be sending you samples of human A- SO_3 , B- SO_3 and recombined
 insulin soon for your assays. I want to say how much I enjoyed talking
 to you, Irv, and Paul.

In my absence, Ron Wetzel will be working on the CNBr cleavage problem
 and Dan Yansura on the purifications of A and B. I will see you in
 February. Have a happy holiday season!

With Best Reagrds,

Michael J. Ross

MICHAEL J. ROSS PH.D.

cc: Irving Johnson
 Paul Bennett

MJR/em

Project: Cloning and Expression of the VP₃ Protein of Foot and Mouth Disease Virus

(Cooperative recombinant DNA project with the USDA Plum Island Animal Disease Center, Greenport, New York—principal investigator, Dr. Howard Bachrach, and Genentech, Inc., South San Francisco, California—principal investigator Dr. Dennis Kleid)

Stage IV: Construction of and safety testing of plasmids derived from various FMDV strains.

Part I: Plasmid Construction and Analysis

A. Cloning of Several FMDV Strains in Subgenomic Fragments

The genome of the FMD Virus is an RNA molecule approximately 8000 nucleotides in length containing a protein covalently attached to the 5' end and a polyadenylic acid sequence at the 3' end. FMD Viral RNA from four strains, which we will call A, B, C, and D, were annealed separately with oligodeoxynucleotide primers to form primer template complexes. The oligonucleotide primers used were: (1) oligothymidylic acid (dT₁₂₋₁₈), dT primer; (2) a synthetic oligonucleotide (dS primer), complementary to a site approximately 5000 nucleotides from the 5' end. The DNA sequence was derived from a cloned FMDV DNA fragment previously obtained and sequenced at Genentech.

Double stranded DNA fragments were synthesized from the various primer-template complexes: A+dT, A+dS, B+dT, B+dS, C+dT, D+dT. Two other complexes, C+dS and D+dS, did not give suitable quantities of ds cDNA. Subgenomic size fragments of 1000-3000 base pairs were isolated by polyacrylamide gel electrophoresis, then treated to give single-stranded polydeoxycytidylic acid attached to 3' terminal ends, using standard procedures (Goeddel *et al.* Nature 281, 544 [1979].) These fragments were annealed to the plasmid pBR322 (previously treated with the restriction endonuclease Pst I and a DNA polymerase to produce polydeoxyguanylic acid the 3' terminal ends). *E. coli* K12 cells were then transformed with the DNA preparations, and grown on petri plates containing suitable antibiotics.

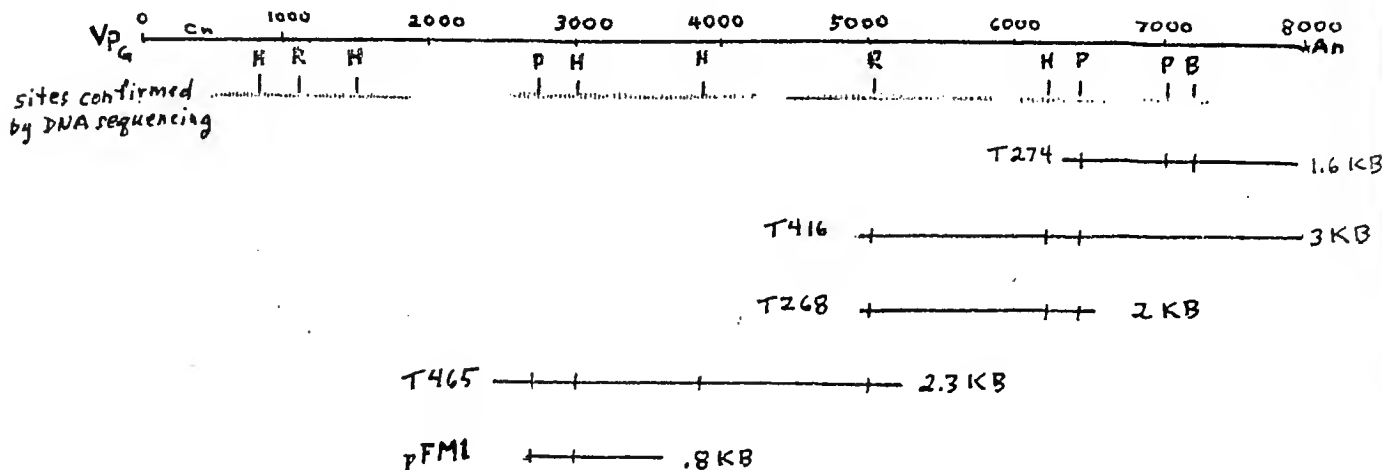
A large number of colonies (1600) were picked and grown in individual 3 ml cultures and plasmid DNA isolated. 500 were from the A+dT synthesis, 300 from A+dS, 200 from B+dT and 200 from C+dT and 200 from D+dT. Each of these plasmids was examined on an agarose gel to determine the size of the insert. No inserts greater than 3500 base pairs were found.

B. Characterization of Plasmids and Inserted DNA Molecules

(1) Hybridization Data

Using nucleotide sequence and restriction enzyme analysis data obtained from the previously cloned A₁₂ strain, we were able to map a series of clones from the A+dT primer synthesis. The plasmid inserts form a family that extends from the 3' end to a site approximately 3000 nucleotides from the 5' end. We found that some of the cloned inserts (approximately 20 percent) do not contain

nucleotides from the 3' end. (This result was also recently reported by Küpper *et al.* *Nature* 289, 555 [1981].) An analysis of the inserts gives the following map:

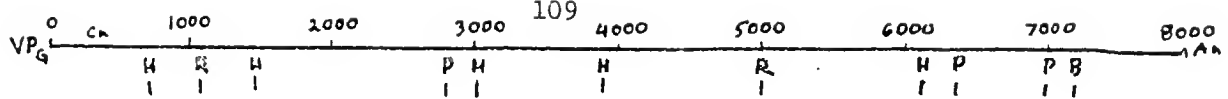


A + dT primer

Using restriction endonucleases a DNA fragment was cleaved from the plasmid T465, radioactively labelled and used as a probe. We determined the DNA sequence of this fragment and proved its location by comparison to known amino acid sequence data (Bachrach *et al.* *Intervirology* 12, 65 [1979].) (The fragment was also subcloned into a pBR322 plasmid to form pFM₁.) Samples of all of the plasmids from the different strains, that were longer than 1000 nucleotides, were spotted on nitrocellulose filters and hybridized to this probe using standard methods (Grunstein and Hogness *P.N.A.S* 72, 396 [1975].) Plasmids containing inserts from this area of the genome were revealed by this technique since the various FMDV strains share greater than 80 percent nucleotide sequence homology. Plasmids containing sequences exclusively from the 3' end of the genome did not hybridize to the probe.

(2) Restriction Analysis Data

We have used the restriction enzymes Hae III, Hpa II, Eco RI, Pst, Ava I, Hind III, Bam HI, Pvu II, and Hinf, to determine which of the inserts overlap each other and create a fairly accurate restriction map for most of the FMDV genome. A summary of that data for each of the FMDV strains is shown below.



T465 ————— 2.3 KB

A100 ————— 2.2 KB ✓

A1 ————— 2.2 KB ✓

A214 ————— 2.5 KB ✓

A281 ————— 2 KB ✓

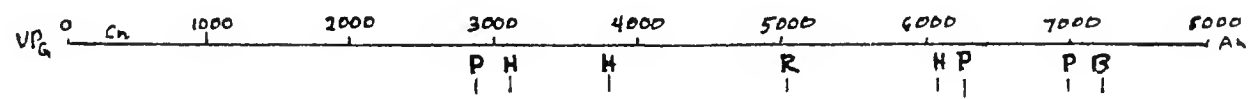
A173 ————— 2 KB ✓

A202 ————— 2.5 KB

A200 ————— 1.5 KB

A + dS primer

Note: plasmids A202 and A200 do not extend beyond the poly C tract. This was determined by nucleotide sequence analysis of the previously cloned pCT220 which terminates at the poly C tract, and restriction analysis of A202 and A200.



B311 ————— 2 KB

B255 ————— 2.5 KB

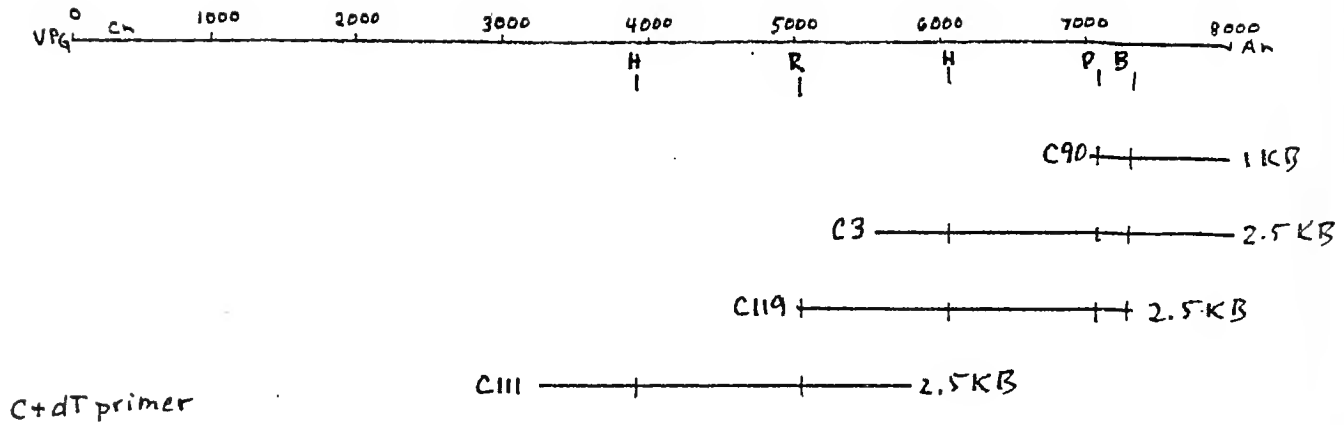
B211 ————— 3 KB

B + dT primer

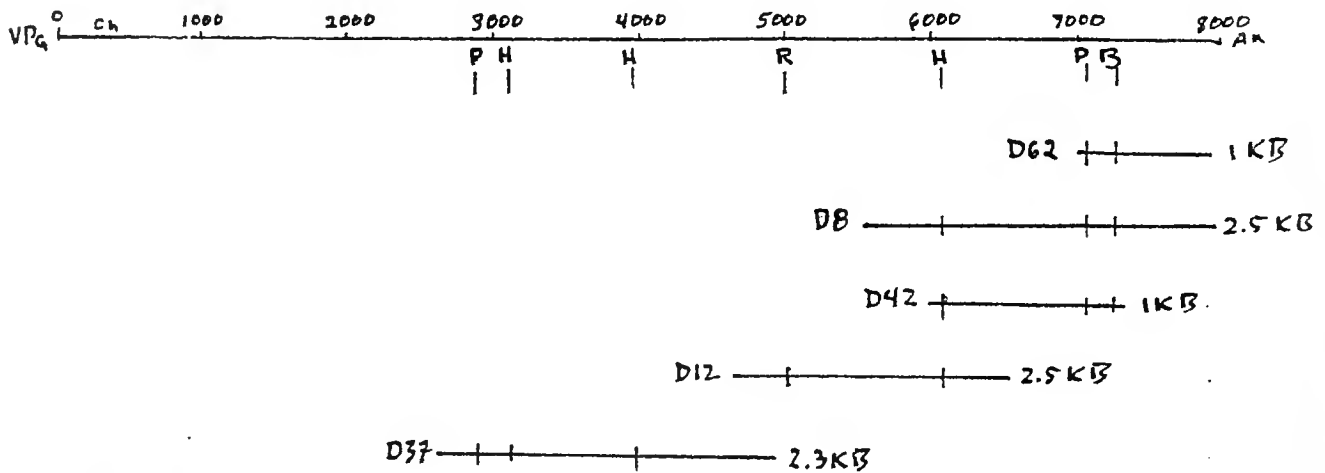
B68 ————— 2.2 KB

B119 ————— 2.2 KB

B + dS primer



C+dT primer



D+dT primer

Using this data we can divide the plasmids into five classes:

- class 1, the extreme 5' end none
- class 2, near the 5' end A200, A202 ;
- class 3, near the middle of the genome
(hybridize to the T465 probe)
T465, pFM1, A100, A1, A214, A281,
A173, B68, B119, C111, D37
- class 4, between the middle and the 3' end
(missing approximately 1000 base pairs from the 3' end)
T268, B255, B211, C119, D42, D12
- class 5, near the 3' end
T274, T416, B311, C90, C3, D62, D8

(3) Summary of Data

We have two independent lines of evidence that show that the plasmids in class 5 contain sequences from the 3' end. (1) plasmids containing cDNA inserts primed by dT primer are predominately from the 3' end (approximately 80 percent). (2) We have determined the detailed restriction map of several examples of 3' end inserts from each FMDV strain. For each strain these tests show unique fragments that are from the 3' end. All other plasmids from classes 1-4 are missing those unique sites and have instead other unique restriction patterns not present in 3' end inserts. These data are consistent with the hybridization results.

Part II: Safety Tests

Studies showing that the plasmid preparations are free of virus contamination and innocuous are now in progress. These tests are standardized and similar to those done by the USDA previously for plasmid DNA samples. These tests are expected to be completed by May 14th.

There are a total of 26 plasmids in the collection. Each DNA sample preparation was diluted into 1 ml of buffer and 200 μ l sample dispensed into individual ampules and sealed. One sample of each plasmid was combined into a 5 ml solution that we used for safety tests.

Part III: Transfer of Plasmids to Genentech

The NIH RAC stipulated that "clones to be approved for removal from Plum Island shall not contain among them, collectively or individually, the full genome of the Foot and Mouth Disease Virus." (Federal Register Vol. 45, No. 12 1/17/80 p3552-3556)

We have agreed to leave all class 5 plasmids, those that contain sequences from the 3' end of the genome, on the island. We have also found that none of our inserts contain sequences from the 5' end of the genome, beyond the poly C tract, (note: the plasmid designated pCT 29 in the June 1980 report turned out, as determined by DNA sequence analysis, not to be from the extreme 5' end but terminated 3' to the poly C tract, some 400 nucleotides from the 5' end.)

We would like to request approval for transfer to Genentech, upon completion of the safety tests, of the following plasmids:

Strain A, T268, T465, pFM1, A100, A1, A214, A281, A173, A200, A202

Strain B, B255, B211, B68, B119

Strain C, C119, C111

Strain D, D42, D12, D37

We have shown that the cDNA inserts contained in the plasmids do not contain sequences derived from the 5' or the 3' end of the genome of Foot and Mouth Disease Virus.

GENECOUN, INC.*



* *GENETIC COUNSELING (Budget Rates)*

Genecoun, Inc., 1980

(left to right: Dan Yansura, Greg Weddell, Maureen Hoatlin, and Don Dowbenko)

We were working on Plum Island in the P4 lab, when Doug Moore, one of the leading USDA scientists working with us, dropped in and wanted to take a picture of us. Of course we immediately saw a chance for a few laughs, something that was always welcome when working under the prison-like conditions there. So we did what we could with the fake cigarettes. The uniforms are standare fare provided by the government facility, as anything personal that you took into the labs could never come out. Usually we went to Plum Island in groups of three or four, for two to three weeks at a time, to clone the foot-and-mouth VP3 genes. Apparently the "Genecoun, Inc." caption provided by the USDA scientists alludes to some lowlife enterprise--genetic counseling at budget rates. The USDA scientists had a good sense of humor, and we enjoyed interacting with them very much. [DGY]

NEWS

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Block Announces Production of Foot and Mouth Disease Vaccine

[1981]

SACRAMENTO, Calif., June 18, Secretary of Agriculture John R. Block today announced a breakthrough in genetic engineering to produce a vaccine against the virus of foot and mouth disease, one of the world's most serious animal diseases.

"This breakthrough can mean annual savings of billions of dollars and an increase in the world's supply of meat," Block said.

We believe this to be the first production through gene splicing of an effective vaccine against any disease in animals or humans. Animal tests carried out over an eight-week period ending today show that the vaccine works," he said.

Block said the breakthrough was in the application of "recombinant DNA technology," a form of genetic engineering whereby a single gene or small series of genes from one organism are inserted into the DNA of another organism.

The work was done under a cooperative agreement between the U. S. Department of Agriculture's Science and Education Administration and Genentech, Inc., a San Francisco-based research firm.

USDA and Genentech scientists carried out the tests as well as the developmental work on the vaccine, at USDA's high containment facility at the Plum Island Animal Disease Center, about 1-1/2 miles off the coast of Long Island, N. Y.

Genentech handled non-hazardous aspects of the work at its California facilities.

"Foot and mouth disease is a highly contagious disease of cattle, sheep, swine and many other animals", Block said. There is no known cure. When an outbreak occurs, exposed and infected animals must be destroyed. Although outbreaks have occurred here in the past, foot and mouth disease does not now exist in the United States.

"The vaccine produced by the new recombinant DNA technology is safe and effective. It cannot produce the disease in a vaccinated animal because only a segment of the virus is used, not the whole virus. Also the vaccine produced with the new technology can be stored for long periods of time without refrigeration. It is economical to produce and greater quantities can be produced at a time than was possible under previous methods of production."

FACT SHEET
RECOMBINANT DNA RESEARCH
FOOT AND MOUTH DISEASE.

SEA and Genentech scientists have reproduced, through gene cloning, a fraction of the foot and mouth disease virus coat. The fraction, called VP₃ is one of four major proteins (VP₁, VP₂, VP₃, VP₄) or polypeptides in the foot and mouth disease virus coat. Biochemist Howard L. Bachrach and research colleagues at Plum Island demonstrated in 1975 that the sub-unit VP₃ is non-infectious but capable of producing immunity in livestock. However, until the new recombinant DNA techniques were developed production of the polypeptide VP₃ vaccine was not possible on a commercial scale. It had to be produced through conventional methods from purified inactivated virus. These techniques are time consuming and costly.

These production methods and others used to produce whole virus vaccines are risky. If the virus is not properly inactivated, the vaccine could cause the disease in vaccinated animals. An escape of the live virus from the laboratory is always possible.

Nevertheless, more than 500 million doses of whole virus vaccines are currently produced and used annually in countries where foot and mouth exists.

In the recombinant DNA production methods, scientists use the bacteria Escherichia coli, Strain K-12, as the host for reproducing the VP₃ polypeptide of the foot and mouth disease virus coat. Using a cutting enzyme, scientists

cut apart a plasmid (small ring of DNA) from the E. coli bacterium. Then they isolate the VP₃ DNA fragment, splice this DNA fragment into the E. coli plasmid, and insert the recombinant plasmid into the E. coli bacterium. The bio-engineered plasmid can then be cloned in the bacteria to produce the foot-and-mouth disease vaccine.

Yields of the immunizing protein, VP₃ obtained by this technique are such that production of commercial quantities of the vaccine are feasible.

Production of the VP₃ subunit in the E. coli bacteria, in the work reported today yielded approximately one million or more molecules of the immunizing protein per cell. In earlier reported recombinant DNA experiments in Germany, bacteria produced only 1,000 molecules of protein per cell. Tests on the protein for immunization potential were not reported. Work in England reported molecular cloning of nucleotide sequences corresponding to the protein genes of foot-and-mouth disease virus.

The National Institutes of Health, through its Recombinant DNA Advisory Committee, establishes guidelines for recombinant DNA research. Permission for the USDA-Genentech project and step-by-step approvals were obtained from the Recombinant DNA Committee and the work was continually monitored by a specially appointed committee.

The cooperative agreement between USDA and Genentech involved no exchange of money. Genentech scientists in effect "invented" the recombined plasmid, from which the VP₃ vaccine can be produced, through cloning. Therefore, the

company has patent rights and the right to license the manufacture of the vaccine. USDA, however, retains the right to make use of the "invention" without payment of royalty, at any time there is a need in this country.

*American Association
for the Advancement of Science*

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January 12, 1983

Dr. Dennis G. Kleid ✓
Dr. Douglas M. Moore

Gentlemen:

Congratulations on the selection of your paper, "Cloned Viral Protein Vaccine for Foot-and-Mouth Disease: Responses in Cattle and Swine," for the 1982 AAAS Newcomb-Cleveland Prize.

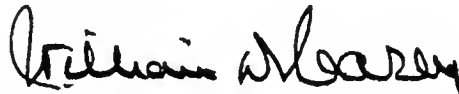
You and the other eight authors will each receive a bronze medal, but I need your advice on how the \$5,000 prize should be divided. In ten equal parts?

The presentation will take place during the Association's 1983 Annual Meeting in Detroit at a brief ceremony preceding the AAAS President's Public Lecture. The time is 8:30 P.M. on May 29; the place is the Renaissance Ballroom of the Westin Hotel in the Renaissance Center. I hope you can be there to receive the prize in person. AAAS will be pleased to reimburse each of you for the cost of one round-trip air fare (coach rate).

Please let me know whether you can be with us on May 29 and whether you will be accompanied by family members. We will want to reserve seats for you and for them in the front row.

I also look forward to hearing from you about the division of the prize money.

Sincerely,



William D. Carey
Executive Officer

WDC:lm

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Sally Smith Hughes

Graduated from the University of California, Berkeley, in 1963 with an A.B. degree in zoology, and from the University of California, San Francisco, in 1966 with an M.A. degree in anatomy. She received a Ph.D. degree in the history of science and medicine from the Royal Postgraduate Medical School, University of London, in 1972.

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Presently research historian and principal editor on medical and scientific topics for the Regional Oral History Office, University of California, Berkeley. Author of *The Virus: A History of the Concept*, Sally Smith Hughes is currently interviewing and writing in the fields of AIDS and molecular biology/biotechnology.

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