Dr. Patricia Ann Rosa
Oral History
January 12 & 17, 2024

Patricia Rosa, Ph.D., 2005

This is an oral history with Dr. Patricia Ann Rosa on January 12 and 17, 2024, about her career at the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH). The interview is being done over Zoom and the interviewer is Victoria Harden, Founding Director, Emerita, of the Office of NIH History and Stetten Museum, National Institutes of Health.

Harden: Dr. Rosa, would you please state your full name, that you know that this interview is being recorded, and that you give permission for the recording?

Rosa: My name is Patricia Ann Rosa. I acknowledge that this is being recorded and that is with my full permission.

Harden: Thank you. You were born on June 21st, 1954, in Fort Bragg, California, the youngest child of Paul Rosa, a logger and rancher, and Rowena Rosa, an advertising agent, housewife, and mother. Your

brother, Richard, was four years older, and your sister, Paula, was just one year ahead of you. Would you tell me about your life growing up through high school, especially about any family members or teachers or others who nudged you towards a career in science?

Rosa: I was born in Fort Bragg, a small town on the coast of Mendocino County in Northern California, where my mother and father had grown up. When I was born, we lived in an isolated logging camp called Wolf Creek Timber Company, located several hours north of Fort Bragg. This site, officially Wheeler at Jackass Creek, is currently part of the Sinkyone Wilderness State Park on the Lost Coast of California, accessible by foot on a coastal trail.

Wolf Creek, Mendocino County, CA, looking down at lagoon & black sand beach from house site.

The logging camp at Wheeler was started as a business venture in the late 1940s by a group of individuals who hired my father as a faller and subsequently as the woods boss for their logging operation. This site was chosen, despite its remoteness and rugged terrain, because of the surrounding old growth redwood trees. Once established, maintaining the logging operation at Wolf Creek was an ongoing challenge due to the winter storms that damaged the mill and washed out the roads. I was quite young when we lived there, but I have idyllic memories of Wolf Creek because it was such an isolated, vibrant, self-contained community. My mother and father had known each other when they were growing up in Fort Bragg, but they had gone separate ways after high school. They had both been married and divorced when they reconnected after World War II. My mother was working in San Francisco, when she married my father and joined him in a remote logging camp.

Before going into labor with my sister and me, my mother stayed with her aunts in Fort Bragg to be close to the hospital, while my father continued to work in the woods. Wolf Creek had a one-room

schoolhouse with first through eighth grades. The kind and excellent teacher, Mrs. Ethyl Dundore, was a widow whose brother, Art Bashore, built and maintained the logging roads at Wolf Creek. Juanita Bashore, Art's wife, was a nurse who vaccinated the children and took care of minor medical needs in the logging camp. The older students had to drive to meet a bus that took them to the closest high school in a small town called Leggett, where Highways 1 [California Route 1] and 101 merge.
When I was 5, my mother would take me to the schoolhouse on Friday afternoons to sing with the other children. I had no official preschool or kindergarten instruction, but I remember my brother and sister, particularly my brother, giving me things that I would pretend to copy. My brother's instruction continued as we grew older, whether it was playing sports, science class lessons, catching fish, or building forts. He was (and still is) a wonderful brother who always wanted his sisters included in whatever he was doing.

I was six when we moved from Wolf Creek after a powerful winter storm decimated the lumberyard, sawmill, and much of logging operation, resulting in the business decision to close the camp. At that point, my father and his crew continued to log at a different site near the coast, but we moved as a family to a pear and walnut ranch in Lake County, a secluded valley in the coastal range directly north of Napa. I started first grade in Lakeport and went through public school there with my brother and sister, all of us graduating from Clear Lake High School.

None of us initially liked Lake County very much, primarily because we were not by the ocean. When we were in Wolf Creek, we lived in a house overlooking a lagoon and a very beautiful black sand beach. Suddenly, we were inland, not with people we knew, and it was hot in the summertime and colder in the winter. My mother was rather begrudging about the move because she had no desire to live on a ranch. My father continued to log with his crew on the coast, which meant he left early Monday morning and returned early Friday evening. Mama was the overseer of anything that needed to happen in the orchards during the week. And when my father came home, he worked all weekend, completing what other people would have done during the week. He was a very industrious, hardworking man, and I hardly knew him growing up because he was physically not present, or he was busy working on the ranch. My mother was great—I would not say she was sweet, but she was a loving mother. Raising children was not necessarily what she was cut out to do. She was the only child of older parents, with many aunts and uncles but no younger cousins or siblings. She loved us, but she had no desire for us to be younger than we were. I think she probably related to us more as peers rather than as parent and children. Our friends liked and respected our parents and continued to visit them even when we no longer lived at home.

Although I did not learn to read before first grade, I quickly became a bookworm. I spent a lot of time reading or playing outside with my brother and sister. School came pretty easily, and I do not remember stressing over schoolwork. In terms of significant people, I had two fabulous science teachers. In seventh and eighth grades, instead of just having one teacher for all subjects, we had a homeroom teacher and then we moved around to different classrooms. A teacher named Mr. Mickey A. McLeod taught a course that I think was called "life sciences," which was an introduction to basic science, as well as psychology, behavior, health—all manner of things. What was most impressive was how much Mr. MacLeod loved life, embraced it, and conveyed his enthusiasm for everything around him. He had a serious limp from

polio, but it was irrelevant because there were so many other things in the world that really mattered to him.

Also, at that point (in middle school), I found that I loved language. We had learned a smattering of Spanish vocabulary words in elementary school as part of the California public school curriculum, but we started to formally study Spanish in seventh and eighth grades. I really enjoyed learning a foreign language and it came pretty easily. Between eighth grade and my freshman year of high school, our Spanish teacher, Mr. Robert McAlear, introduced us to an exchange program with students in Mexico. It was an informal arrangement—he knew somebody who organized it—rather than an official program through the school. I was happy to do it because I thought, "This will be fun. It will be an adventure," but I had no idea what I was doing. My parents allowed me to go because I had lived a pretty sheltered existence as their youngest child and was perhaps too attached. My father's parents immigrated as adults from northern Italy and my mother's grandparents were German, Norwegian and Danish. As children of immigrants, they had been taught that you needed to be able to get up and move off on your own in order to get ahead. I would not say that they were pushing me, but they were pleased that I wanted to go away for the summer.

I was the only student from my school to participate in the exchange program and in retrospect, it seems loosely organized. My mother drove me to a hotel parking lot in San Jose, where I got into a camper on the back of a pickup truck along with a number of other kids whom I did not know. The man in charge of the exchange program then just drove off and delivered us to families in western Mexico. I was going to stay with the Ramos family in Navojoa, Sonora, and because there were other kids to deliver, the driver wanted to drop me off in the town square even though there was nobody there to meet me. I told him, "I think you better take me to the house." When we got there, I found out that the family had not even expected me for another two days, so it was quite an unsettling arrival.

The first thing I quickly realized was how homesick I was. I had never been away from home, so I did not know what that meant. I was also bored. It was a big house, a big family, and they were fairly well-off, so they had servants and there was nothing for me to do. I also realized that my brother, who had just graduated from high school, would be going off to college and I would not get to see him when I got home, which added misery to my homesickness. Ultimately, I persuaded the Ramos family (because they were aware that I was unhappy) to take me to their ranch in Chihuahua, on the other side of the coast ranges. They were hesitant because the ranch was very primitive, without running water or electricity, but there were horses to ride and things to do, which made me happy. After we got to the ranch, a series of torrential rains made the road too muddy to easily return to Navojoa, so we stayed there until it was time for me to go back to California at the end of the summer.

This meant my parents went from getting a lot of very homesick letters to no communication at all from me. My mother just knew that she had to return to the parking lot of the same hotel in San Jose on a particular day to pick me up. She was relieved and amused when I emerged from the back of the camper, oddly stained with the dyes of a colorful serape, but well and happy. This exchange program was a good experience in many ways, but mostly it made me realize that happiness does not completely depend upon your physical surroundings. It has to do with being engaged and active and enjoying things. Despite how much I missed and loved my family, this was the first time I had lived anywhere or seen anything

other than a relatively comfortable, but not affluent, middle-class American existence. I remember waking up after we had crossed into Mexico during the night on the trip down, looking out the window, and seeing poverty, seeing people standing by the road begging. These were things that I had read about but had no firsthand appreciation of them. That kind of experience, I think, was a good thing for me to hold onto—not that I can necessarily do anything about it or have done anything about it, but just to realize that so much of what I have came by virtue of where I was born, nothing that I contributed to, just the luck of it. I am very grateful for what I had been given.

High school was the first time I really thought about consciously working hard. I also had a fantastic science teacher in high school, Mr. Jerry Hendricks. He was from a local ranch family and had lost a hand in a riding accident, which didn't deter him from anything. He taught chemistry and physics, cackling with delight when stumping us in chemistry class. My favorite experience in high school was solving for unknowns in his inorganic chemistry class, in which you had to apply what you had learned in order to properly identify the composition of various inorganic substances. The end-of-year exam was graded based on how many unknowns you could correctly identify. I think that was probably the first time I realized how much I loved solving puzzles, which is probably what has kept me enticed by science all along.

I have not yet mentioned my sister Paula, who was just a year older than me. She and I were very close growing up and still are. However, we were very different and spent most of our childhood arguing with each other. We were often grouped as Patti and Paula, or Paula and Patti, and mistaken for each other, which annoyed us. Our father, who built the house that we lived in, created a large bedroom for us with three doors, and stated that he would only put a wall down the middle when we learned to coexist without fighting, which never happened. We just continued to share the room and grumble. We never had serious fights, obviously. We did everything together, but we could disagree on just about anything. I am sure it annoyed our father, who was irrationally irritated by random noises. I can sympathize, because I am also bothered by random noises, as is one of our daughters. I learned recently that this is a genetic trait that I presumably inherited from my father and passed on to a daughter. I remember a time as a child when we were eating lunch together and my father looked at us and said, "Could you possibly make any more noise chewing your carrots?"

My father was very musical; he had perfect pitch and loved opera. I remember him pruning the pear trees—I guess it would have been late winter when the woods were closed because it was too wet—and listening to the San Francisco Opera on a transistor radio while he was standing on a ladder. I think it is probably part of having a relatively good ear and not being able to stand the random noise.

Harden: You graduated from high school in 1972 as a Governor's Scholar. Can you tell me what that honor meant?

Rosa: I think it just meant that I had straight As and did well on standardized tests. I was also class valedictorian when I graduated from high school.

Harden: That explains why you got into such a fine university. Why did you choose UCSD [University of California, San Diego]? The weather in San Diego is delightful, the school is highly rated, but so are many other universities. So tell me about this transition from high school to college.

Rosa: There was never any question when we were growing up that if we were capable, we were going to go to college. My father went to a mechanical school in Los Angeles and then returned to northern California to work in the woods before being drafted into the medical corps of the Army in WWII. My mother went to Santa Rosa Junior College and then to work in San Francisco. I believe she wrote advertising copy for a local department store (Joseph Magnin) and a hat company (Consolidated Milliners). College was an opportunity that we had, which was not available to our parents. To my mother's credit, she probably played a bigger role in my getting into the college I wanted to attend, and also for other kids in my class, than the school counselor, because she was the one who paid attention to the University of California admission deadlines and standardized test dates. She was president of the PTA [Parent Teacher Association] for many years and very much involved in making sure that the education we received through the Lakeport public school system, although small and in a rural area, was as good as possible. And we did have excellent teachers!

I think part of the reason I decided to go to UC San Diego was because I was interested in marine biology and knew that UCSD was affiliated with the Scripps Institute of Oceanography. Then there was the fact that my sister Paula had gone to Berkeley [University of California, Berkeley] to study physical anthropology, so I wanted to go to the other end of the state. And I was definitely going to a University of California school, because it was cheaper than a private university, and we took advantage of that. We never considered going to Stanford [Stanford University], USC [University of Southern California] or to any other private or out-of-state school because of the expense. You could get a really fine education through the State of California in the University of California system.

I had never been to UCSD before I applied and was accepted. My sister, mother, and I drove down I-5 [U.S. Interstate 5 highway] to visit UCSD during spring break of my senior year in high school. The central portion of I-5 had just been completed, connecting the San Francisco Bay area with the Los Angeles basin along the western side of the San Joaquin Valley. It was much more direct than the coastal route, but I hated it because it seemed like a big, blank, ugly, hot valley. La Jolla, however, was lovely when we finally got there. I had an expectation of the gorgeous old buildings of the Cal Berkeley campus and Stern Hall, the beautiful women's dorm in the Berkeley Hills where Paula lived, but UCSD was completely different. It was a cinder block campus on a plateau above La Jolla without anything else around it. It was pretty shocking initially, but science wise, it was fabulous. What I really appreciated about it at the time, and since, is that UCSD was very young in 1972. There were only three colleges, and it was quite small. I was in Revelle College, which was for hardcore science majors, but the curriculum was designed to provide a liberal arts education. Everybody took the same courses during the first two years regardless of their major. This included two years of a humanities series and a social science component. There was a foreign language requirement, which I tested out of with Spanish, but I took French and minored in Spanish literature. I really enjoyed the humanities series because it encompassed literature, philosophy, and history, and the focus depended on the professor who was teaching the class. We started back with the Greeks and Romans during the first quarter of freshman year and made our way forward until the final quarter of our sophomore year. I was initially intimidated by the math curriculum, because my high

school math had only taken me through trigonometry and I was concerned about calculus because my brother had taken it in college and really floundered. I thought, "If my brother cannot do it, it must be horrible." But in fact, it was very doable. I recently read that the best way to learn calculus was just to work through it, and that was exactly the way calculus was taught at Revelle College in 1972. The textbooks we used were not even published yet, but the man who wrote them was our math professor. You worked through a section of the textbook, took an exam, and moved ahead to the next section only if you had successfully mastered the previous section; your grade at the end of the quarter came from how far you got in the workbook. I had two years of higher-level math at UCSD, which I was proud to have completed, although I had no desire to be a mathematician. I had to work really hard during the first 2 years at UCSD (I wanted to maintain a 4.0 GPA), but it made me realize that mastering something that seemed so challenging at the beginning was really just a matter of working through it. I was not a brilliant mathematician, but there was nothing that I could not do if I worked at it. I took the same approach with physics.

Harden: You majored in biology, graduating with highest honors in 1976. Tell me about your decision to major in biology and if you did any particular projects that excited your interest.

Rosa: At Revelle College, I arrived thinking I might major in marine biology, but this was when molecular biology had just come of age, and that was the focus of biology at Revelle. As a biology major, I did not take anatomy, physiology, botany, or cell biology courses, and by the end of my sophomore year, the only basic biology class I had taken was an introduction to molecular biology, which I loved. I had a good advisor, Curt Stern [Dr. Curt J. Stern]. I talked to him because I had done something with marine biology and realized that I did not find it very interesting. I remember that he said, "You can always come back and study something molecular in the ocean if your real attachment is to it rather than marine biology."

Harden: Tell me more about your professors. You just mentioned one. And feel free to name them.

Rosa: I cannot say that there was one especially important professor during my undergraduate years. Dr. Shenk [Dr. Norman A. Shenk] was the man who taught our calculus class and who wrote the books we used.

Perhaps the most significant experience I had as an undergraduate was my junior year abroad at the University of Edinburgh, through the Education Abroad Program (EAP) of the University of California, which is still going strong. I wanted to study abroad in a place where I could be comfortable taking the requisite science classes. The standard curriculum got very heavy in the third year at Revelle College: organic chemistry, physical chemistry, biochemistry, and upper-level biology classes. I had always had an infatuation—without having been there—with Scotland. The University of Edinburgh participated in the EAP and, provided I did well, the science courses I took there would be accepted by the University of California. So I went there for my junior year and after a brief stint of homesickness, I loved it. That was the first time I was introduced to the developmental biology. There was a fabulous professor, D.E.S. Truman [Dr. D.E.S. Truman], who was a developmental biologist and also an epigeneticist. Developmental biology is the study of how something goes from a single cell to a higher-level organism, and epigenetics is the study of how the environment influences how genes work. It was fascinating.

The system in Scotland is very different from what I described at UC San Diego. They have two terms, fall term and then spring term, and at the end of the year, there is a month's break when you study, and then you have a final exam that covers everything. Your final grade comes from how you do on these final exams. I crammed a lot of physical chemistry and organic chemistry in order to successfully pass my exams. Along the way, I made a very good Scottish friend, Joan Carlin, who was delightful. She was an English major, and I was working hard in the sciences. She would get me to go do something else on occasion and forget about my studies, which was fun and healthy. I eventually lost touch with her, but a couple years ago, by contacting a lawyer in Edinburgh with the same name as her brother, who had been a law student, I was able to reconnect with her. I have not seen Joan again, but I just read her Christmas letter and hope to get back to Edinburgh and see her fairly soon.

When I returned to UC San Diego, I had fulfilled many of the course requirements for graduation, so I decided to work in a lab and do an independent study. Because of my newfound fascination with developmental biology, I applied to work with a professor named Bill Loomis [Dr. William F. Loomis], who studied a slime mold called Dictyostelium discoideum, or Dicty as he like to call it. Dictyostelium is a single-cell amoeba that can sense when nutrients are limiting and change into a multicellular organism with a base, a stalk, and a fruiting body. Thus it can develop from a population of freely swimming, identical amoeba into an adherent organism composed of differentiated (non-identical) cells. Another professor at UCSD, Richard Firtel [Dr. Richard A. Firtel], was creating genetic tools for Dictyostelium, which made it possible to study developmental biology in a system that you could also approach using genetics. My project was just growing Dicty under various nutrient-limiting conditions, scraping them up at different time points, making extracts and running enzymatic assays. It sounds terribly boring, I guess it was pretty tedious, but I enjoyed it. There was just something about this process that I liked, in which you were not doing same thing every day, but you were doing something every day in order to try to figure something out. There were also technical details that you needed to improve as you went along. This was my first introduction to basic bench science, and I just loved it.

I graduated a term early because I had enough credits, but I continued to work in the Loomis lab doing research. I also had a job working at a Scandinavian import store in La Jolla, making enough money to pay rent. It was at this time that without really knowing much, I committed to going to graduate school and learning more about how to do science. This was not a terribly conscious decision. It was just, "What else am I going to do?" The biotech industry did not exist. I did not want to be a teacher. I knew I loved working at the bench. And that was what propelled me forward to apply to grad school.

Harden: Let me follow up on that. Was it your decision more than anything else? Did you have mentors, especially Dr. Loomis? Did he say, "Patti, you really are good at this. You ought to go to graduate school"? Did you have that kind of support or not?

Rosa: It is a bit complicated. Bill Loomis was certainly supportive. And I should give my father credit as well. My mother could have pursued her own career, but she chose to be a wife and mother instead. But my father, who was very much the father and the husband and the breadwinner when my sister and I were growing up—it was like, that may be okay for most women—but "You are my daughters. You are going to do something different." One of his defining features was that he did not want his daughters to be what he called "Geisha girls." He had been in the South Pacific during World War II. And the idea that

a woman's sole purpose in life, as artful as it might be, was to give pleasure to men, did not suit what he wanted his daughters to do. I would not say that we were prudishly dressed growing up, but we were never dressed in a way that was designed to accentuate our sexuality. That was just not what we were going to do. We also had a lot of summer jobs, but we were never allowed to work at a hotel or a motel in the resort community. Instead, we had horribly menial jobs, processing and sorting pears in the dry yard or fruit shed, which was extremely boring, but not personally serving anyone. I remember seeing older women still doing this, and thinking, “I would be dead before I would be standing here at their age.” Pretty quickly, I went from the boring stuff to measuring the sugar content of pears to assess their ripeness, and then to the scale house, weighing and recording the incoming loads of fruit behind tractors and outgoing trucks of packed pears. making sure that the weight on each axle was correct. The guy who had done that job before me was the high school math teacher—even teachers had summer jobs. I remember my father asking, "Are you getting the same salary that he was? You are doing the same job; you should be getting paid the same amount." I believe I was.

Patricia Rosa's father Paul, undated.

So opting for graduate school was not a difficult decision. I had done very well grade-wise at UCSD, and I knew I was smart enough. Bill Loomis asked me a pointed question when I came in to interview for the independent study position in his lab. He knew that I played on a co-ed football team at UCSD and asked what position I played. I told him, "I'm the quarterback.” Related to Bill’s question, these experiences growing up helped me realize that I would not be fully satisfied until I was running my own show. It was

clear that that would not happen if I were a technician working in someone else's lab, so that is what propelled me on to grad school.

Harden: Let's talk about your move to Eugene, Oregon, to the Institute of Molecular Biology at the University of Oregon for graduate school. Oregon's weather is lovely, but perhaps not as lovely as San Diego's. Why Eugene? And you studied there with Professor Edward Herbert [Dr. Edward Herbert], I believe, through an NIH pre-doctoral traineeship, which was a prestigious award. So tell me about deciding to go, your financial support, Professor Herbert—just your entire move into graduate school.

Rosa: I did not apply to very many places. I applied and interviewed at Harvard and MIT [Massachusetts Institute of Technology], which I did not like and to which I was not accepted. I had never lived in a real city. La Jolla was a bucolic village by the ocean, with UCSD on the cliffs above it. Eugene was a relatively small college town and felt comfortable. I loved and respected the Institute of Molecular Biology when I was there, and I still do. It was founded in 1959 by three distinguished scientists—Aaron Novick [Dr. Aaron Novick], Frank Stahl [Dr. Franklin W. Stahl], and George Streisinger [Dr. George Streisinger]—as an integration of chemistry, physics, and biology in the burgeoning field of molecular biology. When I arrived in 1976, the Institute was still a small, tight community of people working together, doing basic research and training graduate students and postdocs.

The Institute had received an NIH training grant, which covered the tuition and stipends of graduate students for three years, with a fourth year covered by serving as a teaching assistant in departmental courses. The senior members of the Institute were committed to training young scientists, giving us their time and attention, which was great (and a bit intimidating). The shocking thing for me as a first-year grad student was that I was miserable and not by the fault of the fantastic labs where I was working. My first two rotations were with Frank Stahl, who did seminal work on DNA replication and recombination, and George Streisinger, who pioneered zebrafish as a model organism for developmental biology. When I was an undergrad in Bill Loomis's lab at UCSD, I was lucky to have a daily routine that suited me, but that wasn't my initial experience in graduate school. During my first rotation, I used the same, simple technique every day in order to ask finely detailed genetic questions. During my second rotation, the complexity of the system limited the number of experiments that could be conducted, so most of the time was spent thinking about the experiments that you had just done or were planning to do. These are highly effective and valuable forms of basic research, but they didn't suit me.

I did my third and final rotation in Edward Herbert's lab. Ed had established himself in the nascent field of molecular biology studying hemoglobin biosynthesis at MIT but was not happy there and was recruited to the Chemistry Department at the University of Oregon in 1963, where he became an adjunct member of the Institute. Following a sabbatical at Harvard in 1969, Ed changed the direction of his lab from biological chemistry to neurochemistry, focusing on a small peptide hormone called ACTH (adrenocorticotrophic hormone), which is made in the anterior lobe of the pituitary. As a protein biochemist, the small size of ACTH (39 amino acids) was appealing. Ironically, Ed and his lab subsequently discovered that ACTH is actually derived from a much larger precursor hormone called POMC (pro-opiomelanocortin), which represented a much-heralded scientific breakthrough. Ed's lab was the first to describe what is now a recurring theme, particularly in the endocrine system, that these precursor molecules give rise to hormones with dramatically different activities depending upon how

and where they are processed. My contribution to this project as a graduate student was studying the intermediate lobe of the pituitary, where POMC is processed in a way that does not yield ACTH. The POMC precursor hormone, when cut up by particular cell types in different parts of the pituitary gland, forms endogenous opiates called endorphins and enkephalins. Scientists had hypothesized the occurrence and searched for an endogenous opioid in humans because we have opioid receptors, but prior to the discovery of POMC, all identified opiates were from plant sources or non-human material. Why do humans have a neuro-receptor for a biological component that we do not make? One of the profound outcomes of Ed's research was identifying the endogenous human opioid peptides that we now talk about as endorphins.

I joined Ed's lab through the back door. I decided that wanted to do my third rotation in his lab, or I was going to drop out of grad school because I was just not happy there. I approached Ed to ask if I could work in his lab, and he said, "I'm in the chemistry department, there are two chemistry graduate students who want to join my lab, and you are a biology student, so I do not think I have space for you this spring." When I told a senior graduate student in Ed's lab, Jimmy Roberts [Dr. James Roberts], about Ed's reply, he said, "Patti, just come and work in the lab." Well, I was on a training grant and I was doing some teaching as a first-year grad student. Jimmy said, "Ed won't know since he doesn't have to fund you, and then if you do something good and it works, it will be fine" (Ed's office and his lab were on different floors). And so I joined Ed's lab in the spring of 1977. Jimmy Roberts was also the guy who taught me what I needed to know to work in the lab, which was the type of thing I enjoyed doing. I am not a biochemist, but I was doing biochemistry, I was developing a system. I was doing different things almost every day and loving it.

Harden: Is this the work from which you published your first paper in 1978? That is two years before you received your Ph.D., and I was taken with that and figured there must be something special about what you were doing.

Rosa: Yes, that was it. It was the first thing I did in the lab with Jimmy Roberts and another senior graduate student working on the POMC project. I analyzed POMC processing in the intermediate lobe of the pituitary and compared it to POMC processing in the well-characterized anterior lobe of the pituitary. Their peptide profiles were distinct and my thesis went forward from that.

Harden: So you came into the lab, you did well, and suddenly it was okay and you were a part—

Rosa: Yes. I was funded by the training grant, Jimmy found bench space and trained me, and I was not pushing out any chemistry students. By the time this paper was published, I was part of the lab. Ed was a lovely mentor, he was a totally decent person, he was kind, and he was brilliant. I could not have asked for a better mentor or lab-mates.

Harden: In 1980 you received your Ph.D. in molecular biology, and you moved again, this time to St. Louis, Missouri, for a postdoc in the genetics department that was headed by Donald Schreffler [Dr. Donald C. Schreffler] at Washington University School of Medicine. There you were supported by a Jane Coffin Childs postdoctoral fellowship. Tell me why you chose Washington University and then about what you were doing there.

Rosa: The reason I chose Washington University was because my then-boyfriend and current husband, Paul Policastro [Dr. Paul F. Policastro] had begun graduate school there. He had finished his bachelor and master's degrees at the University of Oregon and was working as a technician in Ed's lab when I joined as a graduate student. Supposedly, he thought, "If she can get a Ph.D., I can get a Ph.D." Paul then applied to doctoral programs and was accepted at Washington University, with the plan that if I got a post-doc at Washington University, we could be together after I finished my Ph.D.

It was established at the outset by the Institute of Molecular Biology that students would complete their doctoral research in four years, or they would receive a Master's Degree as a terminal degree. It was an intense program, and certainly the last six months of graduate school were probably the most stressful of my existence. I had completed the benchwork, but I spent a long time looking at the data and thinking, "How am I going to compose this? How does this all come together as a dissertation?" The requirements for a Ph.D. from the Institute were rigorous. There was an oral diagnostic exam at the beginning of the first year of graduate school, during which committee members identified deficiencies in one's undergraduate education. As a result, I was required to take courses in anatomy, physiology, neurobiology and ecology, among other things. There was also a qualifying exam in the third year that included a mock grant proposal on a topic unrelated to your doctoral research, written and oral presentations of what you had completed and planned to do for your dissertation, and an oral exam to assess your grasp of general and specific scientific knowledge. It was daunting. If you passed the qualifying exam, the fourth/final year was spent completing experiments and assembling a doctoral dissertation, which was an original composition, and not brief introduction and conclusion sections around chapters representing published papers. Personal computers had not been developed and I didn't know how to type, so I wrote my dissertation by hand and paid someone to type it. The dissertation was given to committee members several weeks before the scheduled oral defense, which transpired if they agreed that the dissertation was reasonably well written and merited a Ph.D. If not, one was sent back to revise the dissertation, to conduct additional experiments, or received a Master's Degree if an acceptable outcome was not forthcoming.

To return to your question, I decided to do my post-doc at Washington University Medical School in St. Louis because that's where Paul was. I thought that my anticipated post-doctoral project had a connection to what I had done as a graduate student because it involved hormonal regulation of blood proteins whose levels varied in a sex-limited pattern between different strains of mice. At the time, it appeared to be a model system to study hormonal regulation of protein synthesis, which eventually turned out to be incorrect. I did many experiments and learned a lot about protein secretion in the process, but most of the data I generated were not interesting enough to publish.

It was a rather challenging lab to work in because my mentor, Donald Schreffler, was pre-occupied with fulfilling his duties as Chair of the Genetics Department, a position that he did not want. Don had been recruited from the University of Michigan in 1975 to develop a genetics department at Washington University School of Medicine. He had built the department with the intent that someone else would become chair, but that had not yet materialized. Don was a brilliant immunogeneticist and had demonstrated the multi-gene nature of the Major Histocompatibility Complex (MHC) locus in the early 1960s, while a grad student at Caltech [California Institute of Technology]. Don had grown up on a dairy

farm in Michigan and his acuity as a geneticist became obvious when he was in college. By the time I joined Don's lab at Washington University in 1980, he was a supportive mentor, but his attention was focused on his administrative responsibilities. I realized that I needed to do a second postdoc because I was not sure where my career in science was going. Recombinant DNA technology was just emerging, Paul was finishing up his Ph.D., and I was looking for a second postdoc. My thoughts turned to Southern California, where I had very happy memories of living in La Jolla.

Harden: Before we go there, I want you to answer one question about your research in St. Louis. In 1983, you published with Dr. Schreffler and others, a paper that appeared in the Proceedings of the National Academy of Sciences (PNAS). I will read the long title: "Cultured Hepatocytes from Mouse Strains Expressing High and Low Levels of the Fourth Component of Complement Differ in the Rate of Synthesis of the Protein." It was supported by a grant from NIAID. Tell me about this research and what was so important about it that the paper was published in PNAS?

Rosa: In graduate school, I had learned how to prepare primary cultures, which means you dissect a tissue from an animal and provide what it needs to survive for a limited period of time in culture medium. Learning how to do this was probably the least exciting aspect of my research and it was labor-intensive, but it was an essential component of the experiments I needed to do, both in graduate school and as a post-doc. In Eugene I made primary cultures from mouse and rat pituitary glands, whereas in St. Louis, I was preparing primary cultures of liver cells (hepatocytes) from inbred strains of mice, putatively to study hormonal regulation of two proteins whose blood levels varied among strains and between sexes. Murine hepatocytes synthesize both the fourth component of complement (C4) and a related protein called SLP, the genes for which Don had mapped to the middle of the MHC many years earlier while at Cal Tech. I prepared primary hepatocyte cultures from male and female mice of different inbred strain and analyzed the synthesis, processing and secretion of C4 and SLP. We would now call this secretory processing, looking at where things go inside the cell. The take-home message, as I recollect, was that the differences between strains in the blood levels of C4 and Slp proteins reflected the level at which they were being made by hepatocytes, but there were no sex-dependent differences, with or without added hormones. The complement system comprises a highly regulated cascade of protein synthesis, processing and turnover that provides an important host immune defense against invading pathogens.

By the time I entered the complement field, most of the really exciting, but difficult, groundbreaking work had been completed. Many tools were available and I was doing something meaningful, but not venturing into the unknown. The reason the paper you mentioned was published in PNAS—and I think it was a worthy paper—was primarily because Don Schreffler was a member of the National Academy of Sciences, USA. Would it have been published in PNAS if he had not been an Academy member? Perhaps not.

I had a prestigious post-doctoral fellowship from the Jane Coffin Childs Foundation (JCCF), but I suffered from "imposter syndrome," questioning seriously, "Who am I"? I went to annual JCCF meetings in New Haven where everybody else's mentor was a third generation-scientist from an Ivy League school on the East Coast. I remember the first poster I ever presented. It was something I had put together by hand. I am not artistic, so I used little stick-on letters, and it was quite messy. These meetings were both

inspiring—to be around such smart people—and daunting because of whom I thought I was and what I was able to do.

Harden: The 1970s and the 1980s were, as you have already noted, an extraordinarily fruitful period in biomedical science, especially in immunology with new discoveries being made almost every week. Would you comment in general about this time and how exciting it might have been.

Rosa: It was definitely exciting, especially for immunologists. I think immunologists are a special breed of people. There is a huge amount of knowledge out there and a whole bunch of acronyms. Unless you know what they are, it is as if you are in the presence of people speaking a foreign language. I was not an immunologist, but I appreciated what they did. When I was at Washington University in the early 1980s, there was excitement among immunologists about the I-J sub-region of the MHC, which somehow controlled the generation of immune suppressor cells. Lee Hood [Dr. Leroy E. Hood], a prominent immunologist and molecular biologist, and his colleagues were beginning to sequence large pieces of DNA and immunogeneticists had narrowed the search for I-J suppressors down to a small region of the MHC. However, when Hood and collaborators sequenced across the I-J region of the MHC, there were no obvious coding sequences or genes. The whole field was deflated by this finding. These were not stupid people, they had been rigorously studying a real phenomenon, but when it was inexplicable at the genetic level, everyone thought that I-J must be an artifact. To this day, nobody works on I-J or talks about it. I wonder if I-J can be explained with what's now known about regulatory T cells and modifiers of gene expression.

Harden: Let's drop back just a bit to November 7, 1981, when you married Paul Policastro, who was also a Ph.D. scientist and with whom you had published several articles in Oregon. Tell me a bit about him and how your scientific interests overlapped or differed.

Rosa: I had lived in a small town or a logging camp my entire life. Paul's father was career military, and they had moved around a lot when he was growing up. Paul was the third of eight children and they moved to Europe when he was in middle school. They first lived in Poitiers, then Heidelberg, then Paris, and finally Brussels. Paul's youngest sister was born in Paris. And at some point his father, who was a colonel, transitioned from the U.S. Army to NATO [North Atlantic Treaty Organization]. When I first met Paul, his parents and younger siblings were still in Brussels, and one of his older brothers was working in Heidelberg. They eventually moved back to the U.S. and settled in Northern Virginia, where his father worked for FEMA [Federal Emergency Management Agency] as a logistics specialist. Paul's father had done something with shipping when he was in the Army and had met Paul's mother when he was stationed in New Orleans.

When Paul met me, I think he found me very different but compatible. I had lived in the same place for 12 years while growing up and I lived in a small house by myself in Eugene, with no intention of moving. In contrast, Paul had lived in 8 different houses with lots of roommates in Eugene. We still have common scientific interests but work on different things. We have basic principles in common and we trust each other, which is absolutely essential. And as for my career, I think about being lucky with good mentors, and I was lucky to fall in love with a man who fully supported me. Having had a mother who put her own life aside to follow his father and raise eight children, Paul fully embraced my decision to pursue a career

and have children. Certainly, there are things I am better at, and there are things he is better at, but he was a full and equal parent.

Harden: You have two daughters, Justine and Elise. When they were small and you and Paul still had junior status in your careers, how did you manage what is today called work-life balance?

Rosa: I do not know. We did not have an option. We just did it. But to go back to one thing: their last name. When I was pregnant in the 1980s, we did not know what sex a baby would be before it was born. I had not changed my name when we were married, so we talked about how we would name our children and if we wanted to do "a hyphenated thing." Paul aptly commented, "Rosa-Policastro, how long is that going to last?" So we decided that if we had a girl, she would be a Rosa. If we had a boy, he would be a Policastro. We had two girls, and they were both named Rosa. Elise continued this tradition; she did not change her name when she married and her son has her husband's last name and her daughter's last name is Rosa.

I must give my parents credit for their reserve. My sister Paula and I both marvel at how our mother and father never poked their noses in our personal lives when we were growing up or when we were adults. They supported us, they realized we were going to make mistakes, and assumed we would be smart enough to recognize our errors and move on. I am sure they had plenty of conversations about me retaining my surname and giving it to our daughters, but they never mentioned it to me. It was probably a challenge for our daughters growing up in the small town of Hamilton, Montana, and it was definitely awkward for Paul; if his daughters were named Rosa, he must be Mr. Rosa.

Harden: It is interesting to me how different the narratives are from women and men about all of this, and I thank you for putting this on the record.

Rosa: With respect to your question about what was it like to have two jobs and children: I was a postdoc and Paul was a graduate student when Justine was born. Finding daycare that you could afford and feel good about was challenging. It seemed like I was the only woman at the med school who had a baby, and I was committed to nursing her for six months and not giving her formula because that seemed best. I expressed milk with a breast pump that resembled a bicycle horn. Paul and I managed because there wasn't an alternative. I got a driver's license two weeks before Justine was born although I hated to drive and was not a good driver, but I couldn't expect Paul to be the sole driver because we would need to take turns dropping off and picking up the baby from daycare.

Harden: In 1984, you moved from St. Louis back to Southern California to become a postdoc. This is your second postdoc, as you said, at the Research Institute of Scripps Clinic in La Jolla where you were supported from 1984 to 1986 by a Leukemia Society of America Special Fellowship, and then from 1986 to 1987 by the President's Council of Scripps Clinic Fellowship. From 1987 to 1988, you were a fellow at the Medical Biology Institute in La Jolla. Neither of these institutes—the Scripps or the Medical Biology Institute—are a part of UCSD, so tell me about the institutions and about what kind of private sector research you were doing.

Rosa: By the 1980s, it was called the Research Institute of Scripps Clinic and was located in a relatively new facility adjacent to UCSD and the Salk Institute on Torrey Pines Drive. Prior to that, Scripps Clinic was in downtown La Jolla, where it had been for a long time. We went to Scripps/La Jolla because I decided to do a second postdoc with a scientist named Ronald Ogata [Dr. Ronald T. Ogata]. Ron was a tenure-track member of the Molecular Biology Department at Scripps studying the genetic structure and regulation of C4 and Slp loci, using recombinant DNA tools he had acquired as a postdoc with Wally Gilbert [Dr. Walter Gilbert] at Harvard.

In 1984, recombinant DNA technology was rapidly developing, and I went to Ron's lab because he had these genetic engineering techniques in hand and had decided to focus his attention on the fourth component of complement. I knew about Ron's lab because two people with whom I worked at Wash U, John Atkinson [Dr. John P. Atkinson] and Paul Levine [Dr. R. Paul Levine], were also collaborating with Ron Ogata. Both were very good male mentors who recognized that women needed active support or they would be overlooked. In addition, things were not going terribly well in the lab; Don Schreffler was ill, and I did not want to remain in St. Louis. Paul was in the last year of grad school and I recollected my final year at Oregon and the intense focus that I needed to finish. We decided that it would be a good time for me to move to California with Justine, who was one and a half, get things going in La Jolla, and then he would join us after defending his Ph.D., as he had lined up a postdoc at Scripps as well. So that was how we ended up in La Jolla.

The Research Institute of Scripps Clinic was a very exciting place to work and very well-funded. Paul and I were in the newly formed Molecular Biology Department and Richard Lerner [Dr. Richard A. Lerner], who just passed away in 2021, was the Director of the Institute. Lerner was a very smart scientist and competent administrator. Ron Ogata was also a really smart guy, and we interacted a lot with the adjacent labs of young investigators Greg Sutcliffe [Dr. J. Gregor Sutcliffe] and Tom Shinnick [Dr. Thomas M. Shinnick]. It was a good and lively place to work, and we worked in a beautiful new building overlooking Torrey Pines Golf Course and the Pacific Ocean.

With time, however, the charm dampened. Paul and I had two small children and we were coping with the demands of living and working in Southern California. Ron did not get tenure at Scripps but had become a fellow at a new biotech-related firm, the Medical Biology Institute (MBI) down the road from Scripps. I moved with Ron to MBI and worked there for a year while Paul finished his postdoc at Scripps and we both looked for jobs. I did not know what I wanted to do next.

Harden: You both ended up at the Rocky Mountain Laboratories (RML). This must have been a big deal, deciding to move from the private sector into the federal government. You joined John Swanson's [Dr. John L. Swanson] Laboratory of Microbial Structure and Function (LMSF) as a senior staff fellow, and Paul was also hired at RML and I do not know if he was in Swanson's lab or somewhere else. Please tell me about this transition—did you apply? Or were you recruited? How did it all happen?

Rosa: Luck. We applied for positions that we saw advertised, probably in Science or Nature, but I had no idea about an NIH lab in Montana and knew very little about microbes. I had taken one microbiology class in my senior year at UCSD. I fell asleep while cramming for the final and got the only C in my college existence on the test. I was mortified and wrote a letter of apology to the professor, something

like, "It is not your fault, you told us that we should learn the basics and details would fall into place. But I put off memorizing the Latin names of organisms until the night before the final exam, and then I fell asleep before mastering them." Anyway, I was not a microbiologist and I still do not consider myself a good microbiologist. So our applications for positions at RML were shots in the dark. Paul had been to Montana once, I think, and at least knew that there was a lab in Hamilton. The positions we applied for were with John Swanson, who had been hired in 1979 as Chief of the Laboratory of Microbial Structure and Function at the Rocky Mountain Laboratories (RML) of NIAID, NIH. John had been Dean of Students at the University of Utah College of Medicine and also a professor in the Pathology and Microbiology Departments there. He was a long-standing Neisseria gonorrhoeae researcher. Although John had trained in medicine, he had an affinity for basic research and was at the leading edge of investigations into the molecular pathogenesis of Neisseria, the agent of gonorrhea. He was a tall, large, and abrasive guy, and he was supposedly hired to help keep RML open, because there had been a number of questions raised, like, "Why on earth does NIH have a lab out in Montana, and what are these people doing there?" The historic focus of RML on entomology and vector-borne diseases was very important, but it was not a highly respected topic in the 1970s. Congress wanted NIAID to fund research on topics that were of public interest and in the news, like bacterial pathogenesis. John was hired to bring molecular biology to RML, as well as to "clear out the deadwood." I knew John well, long after he was my boss, and the only regrets I heard him express about his career at RML were related to forcing out scientists who were not doing trendy science, but who were decent and significant scientists. They just were not part of the intramural program that NIAID wanted to support.

Paul and I interviewed separately. I immediately felt very comfortable in Hamilton because I had grown up in a small rural place. Paul had decided when we lived in St. Louis that he wanted to break the habit of moving frequently. When we were both offered jobs and moved to Hamilton, he was happy to be here and to put down roots. In addition to co-workers at the lab, there was also a strong sense of local community, which was something that I had missed. Eugene was a nice college town, but I really did not like St. Louis. I had never lived in a city before. I did not like the weather, and I did not fit into the medical school culture. The situation was also very different when we went back to La Jolla in the mid-1980s. It was bigger and more developed than it had been when I was an undergrad in the early 1970s. We lived north of San Diego in a coastal community that had been engulfed by urban sprawl. We had friends at Scripps, but no sense of local community.

John Swanson, to his credit and to our benefit, hired us although we did not have backgrounds in microbiology. Paul had at least worked on retroviruses as a post-doc, but I had not done any microbial science. Perhaps John thought I would continue to work on complement, which is relevant to infectious disease.

At the end of my time in La Jolla with Ron, PCR [polymerase chain reaction] had just been described, and I had started to use it before there was a thermocycling machine, so I was doing it all manually. I liked to explore new techniques and research fields that had not yet been mined. I realized when I came out and interviewed at RML—and ultimately was hired—that Borrelia burgdorferi was fascinating. It had only recently been described as the causative agent of Lyme disease and not a lot was known about it, which appealed to me. John was fine with my decision to work on Borrelia. Tom Schwan [Dr. Tom G Schwan], who had arrived at RML a few years before us, was a colleague in the Laboratory of Vectors and

Pathogens (LVP), of which Claude Garon was Chief. Tom's assistance made my work possible, because without his knowledge of how to grow Borrelia and his expertise as a vector biologist, I would not have been able to develop a research project at the molecular level.

RML Lab Chiefs and secretaries, 1990

I only learned after the fact that there had been a bit of turf struggle over Borrelia when I decided to work on it. Borrelia had previously been worked on in LMSF, John's lab, and also in LVP, Claude Garon's lab. However, by the time we arrived in 1988, Borrelia research was exclusively done in LVP. I was ignorant of the pre-existing tension between the labs and ultimately, my collaboration with Tom Schwan, who was a very generous and helpful colleague in LVP, helped dissolve the issue.

Harden: Before we get into your research in detail, I want you to describe for me the larger lab, the people with whom you worked in addition to John Swanson, the RML administration, the support staff. Just draw me a picture of how RML operated.

Rosa: The physical plant was part of the original physical buildings. Buildings 1, 2, 3, 4, 5, and 6 formed an E, with Buildings 1, 2 and 3 at the front and the others forming wings off the back. The library was on the top floor and the seminar room was a lovely paneled space with columns in the middle.

When we arrived, there were three labs: John Swanson headed the Laboratory of Microbial Structure and Function or LMSF. Bruce [Dr. Bruce W. Chesebro] was the Chief of the Laboratory of Persistent Viral Diseases or LPVD, and Claude Garron was Chief of the Laboratory of Vectors and Pathogens or LVP, which was a fairly recently formed lab. Claude also directed the electron microscopy branch. RML was much smaller than the Bethesda component of NIAID. Paul decided to work on Rickettsia, the tick-borne agent of Rocky Mountain Spotted Fever, whose identification in Hamilton resulted in the creation of RML in the 1930s. We were hired as Senior Staff Fellows, a position that does not even exist anymore. We had autonomy in what we did, a small lab space and sufficient resources to cover our research. The Staff Fellow program preceded official tenure track programs at NIH and NIAID. It was great for me because if I had suddenly been put in charge of a lab with other scientists under me, I'm not sure I would have known what to do. I love working in the lab, and when we got to RML I could just do my thing for a year, and it worked well. There was someone who handled travel, a person who handled personnel, two guys in the stockroom, some lab techs, and the animal facility. I did not start out doing animal work. There was an in-house machine shop. Everyone at RML was a federal employee, and it was small and highly inter-connected. Administration did not exist as a separate entity. Each lab had a secretary and they were integral components of the lab. The secretaries handled all aspects of our manuscripts, and they were key players in our science. I honestly don't remember if I had a computer, but if I did, I only used it very poorly as a word processor because I can't type.

As I understand, Alan Barbour [Dr. Alan G. Barbour] had originally been hired by John, and he was working partly on Neisseria. There was also another scientist, Herb Stoenner [Dr. Herbert G. Stoenner], who worked on relapsing fever Borrelia, which Alan Barbour also worked on. Willy Burgdorfer [Dr. Willy Burgdorfer] was working on the Lyme disease agent, and Jack Muñoz [Dr. John J. Muñoz], worked on pertussis. When we arrived in June of 1988, a number of senior scientists had retired, Alan Barbour had started his own lab in Texas, and Willy Burgdorfer was retired and an emeritus scientist in Claude's lab. Tom Schwan had been hired, but he was a vector biologist, and supposedly, early on, John Swanson encountered Tom in the hall and said, "You are the kind of guy whom I was hired to fire." However, John and Tom ended up being good friends, and John recognized how my work on Borrelia would not have been possible without Tom as a colleague at RML.

Harden: I understand that John Swanson's organization of his lab was different from, say, Bruce Chesbro's, in that Bruce preferred a traditional lab structure—defined sections headed by PIs—while John preferred to have no formal structure designated, just PIs and their postdocs and technicians pursuing whatever they wished.

Rosa: Yes, definitely. Harlan Caldwell [Dr. Harlan D. Caldwell] was the only other PI in John's lab when we joined. He directed a large Chlamydia program. Harlan soon thereafter became Chief of a separate laboratory, the Laboratory of Intracellular Parasites (LICP). Some of the more junior people, like Ted Hackstadt [Dr. David W. "Ted" Hackstadt], and Bob Heinzen [Dr. Robert Heinzen] left RML to take faculty positions elsewhere, but subsequently returned as tenure-track investigators. Paul began to work on Rickettsia, which along with Chlamydia became part of Harlan Caldwell's LICP.

John Swanson managed the budget and controlled the purse strings, but he was totally generous. If you needed something, you got it. We never worried about money, and he certainly did not spend it on himself. But there was some concern in Bethesda that postdocs and technicians in LMSF had the same autonomy and resources as senior investigators, which was clearly John's way of running things. When questioned about this approach, John responded "cream rises", meaning that good scientists would succeed under their own initiative with minimal direction from him. He offered substantial feedback to junior scientists, but he didn't tell them what to do.

John supported me, gave me a lab, let me work on Borrelia, and things went well. I decided to apply for a Biomedical Science Grant from the Arthritis Foundation to support a technician, which I received. At about this time, a technician in LMSF retired and John said, "You can use this slot to hire a technician and use your Arthritis Foundation funds to hire a postdoc." Which I did, hiring Dan Hogan [Daniel M. Hogan] as a technician with federal funds and Neil Margolis [Dr. Neil Margolis] as a post-doc on the Arthritis Foundation grant. Dan had just graduated from Montana State University, Bozeman with a bachelor's degree in microbiology, and Neil had received his Ph.D. in molecular biology from the University of Pennsylvania in 1990. Things grew slowly, but at a pace that I could handle. First, there was one postdoc, then there were two, then there were three. I think about how relatively junior tenure-track scientists these days immediately have to manage a budget and supervise technicians and postdocs while developing their research project. I would have been overwhelmed.

Harden: Soon after you arrived at RML, you began working with Tom Schwan on an assay for Borrelia burgdorferi, the agent of Lyme disease, and in 1989, the two of you published a paper in the Journal of Infectious Diseases describing a PCR test for diagnosing Lyme disease. Tell me about that work

Rosa: In 1981, before I joined the lab, Borrelia burgdorferi, a spirochetal bacterium, had been discovered as the agent of Lyme disease, with key elements of this work conducted at RML. B. burgdorferi could be isolated from infected ticks through Alan Barbour's and Herb Stoenner's expertise in growing Borrelia in culture medium and Willy Burgdorfer's recognition of what he saw under the microscope in the midguts of ticks. The diagnosis of Lyme disease was problematic. There was not a good test to assess whether someone had serum antibodies that recognized B. burgdorferi and it was extremely difficult to detect spirochetes in the blood of infected animals and people. The tick midgut was a fortuitously unique site where B. burgdorferi replicated to sufficiently high levels for microscopic detection. We speculated that perhaps serum antibodies did not recognize B. burgdorferi when it was in the blood of people or mice because the spirochete looked different in vivo than when it was growing in culture medium in vitro. If that were the case, a polymerase chain reaction (PCR) test for B. burgdorferi would circumvent this problem because it recognizes the DNA of an organism, which does not change with the environment.

When I was at Scripps, I used PCR to amplify the C4 and Slp genes from mouse DNA, whereas at RML I was using it to amplify a specific sequence in B. burgdorferi, a bacterium. This was an inherently easier undertaking because mice have a genome with ~104 greater complexity than bacteria. The needle in the haystack is much easier to find when there is less hay to sort through. A PCR diagnostic test merely requires a target sequence of DNA that is unique to the organism you want to detect and the ability to efficiently and specifically amplify it. In 1988, there were no known sequences of any genes in Borrelia, and cloning and sequencing represented substantial undertaking, which is somewhat hard to fathom today. I started by creating a library of randomly cloned small pieces of DNA from Borrelia burgdorferi and choosing one that was not conserved in a closely related spirochete, Borrelia hermsii, which does not cause Lyme disease. Then I developed the primers and amplification conditions that permitted specific detection of a few Lyme disease spirochetes in a background of a vast excess of mouse or human DNA.

This was the first PCR test for Lyme disease, and in the process of developing it, I realized (and still insist) that PCR is not a good diagnostic assay for Lyme disease, because there are very few organisms present in a persistently infected vertebrate host. After the first week or two of infection, Lyme disease spirochetes are not present in the blood or any other easily sampled human fluid or tissue. They can occasionally be isolated from the margins of the characteristic erythema migrans (EM) skin rash, but this is inefficient, technically demanding, time consuming, and requires a skin biopsy. The spirochete is cleared from the blood by the host immune response and persists in tissues and in the skin at a very low level. In order to diagnose Lyme disease by PCR, you need to sample a big enough piece of skin to have enough Borrelia DNA present to amplify to the level of detection. In humans, this only works if you take skin in the vicinity of the original tick bite, which is often not known, or from the edge of an EM rash, which is not always present.

Anyway, although Tom and I developed the first PCR test for Lyme disease, and it was patented, it is truly not useful as a human diagnostic tool, nor is any other PCR test for Lyme disease. Since PCR amplification is done in a lab where B. burgdorferi is grown and manipulated, and the assay must be capable of detecting just a few organisms, false positive test results due to contaminating DNA remain a serious and complicating issue. Before that shortcoming was widely recognized, there were a number of labs doing very sloppy PCR diagnostics and practically every sample analyzed was positive. This result was used to satisfy patients who believed they had Lyme disease and thereby wanted an extended course of antibiotics prescribed. Eventually, the recognition that infected human and animal hosts mount a robust immune response to B. burgdorferi, generating antibodies that don't clear the infection but severely limit the number of organisms present, led to the development of sensitive and specific sero-diagnostic tests for Lyme disease.

END OF FIRST SITTING OF THE ORAL HISTORY

This is the second sitting of an oral history with Dr. Patricia Ann Rosa, on January 17, 2024, about her career at the National Institute of Allergy and Infectious Diseases. The interview is being done over Zoom, and the interviewer is Victoria Harden.

Harden: Dr. Rosa, from 1993 until 2000, you were a tenure-track investigator. To me seven years seems a long time not to be tenured for someone who had brought recognition to RML via the diagnostic test you had developed and patented and your publications in prestigious journals about the microbiology and genetics of Borrelia burgdorferi. What was going on administratively in your career at this time? Were you an independent investigator? Tell me about all this.

Rosa: Yes, I was an independent investigator. And about 1989 or 1990, as I said earlier, I had a postdoc and then a technician, or a technician and then a postdoc. My lab gradually grew all along, with the caveat, which I always told prospective postdocs, that I did not know what my career was going to be, but even so, they were willing and happy to come to RML and work on Borrelia. The whole tenure-track process, at least as I understood it at NIH, and certainly at RML, had undergone a significant re-definition after publication of what was known as the "Klausner Report" [National Institutes of Health, Intramural Research Program, "Report of the External Advisory Committee of the Director's Advisory Committee, and Implementation Plan and Progress Report" (Bethesda, MD: DHHS/PHS/NIH, 1994)], in which it was recommended that granting tenure be made a rigorous and defined process. Prior to that, junior scientists who got the blessing of their lab chiefs would get additional resources and be promoted in order to become tenured staff members. There was not an official or defined tenure track. Certainly, the NIAID and the NIH promotion and tenure (P&T) committees were real and active, but exactly how you got to appear before that committee was somewhat less defined. I think the reason tenure track began for me in 1993 was coincidental with that change in the policy. My lab chief John Swanson—who may not have been the perfect lab chief for everyone, but for me, he was a very good fit—from the outset was supportive and honest. And that, for me, was and still is extremely reassuring—to know that someone will give me a frank opinion. If I am told that I am doing well, and I know that this is coming from a smart senior scientist, then I must be doing okay. If they tell me, "You could work on this," then it is something that I should put my attention to. Throughout my career with John as my lab chief, and even subsequently when he was a senior friend and mentor, I felt like I always could look to him and respect the advice he gave me. What is critical here is the element of trust that the person has your best interests at heart. I felt like John respected me as a scientist. He knew I was not a microbiologist but that I had other skills and capabilities that were good and that he thought would fit and would work both in his lab and at RML at large. And in reality, the fact that the tenure track process was protracted was a bonus for me in sorting out who I am, gaining confidence that I could do what I set out to do, learning more about Borrelia and microbes, which again, was not my background. So I had no qualms about that.

At that time, lab chiefs had much more budgetary and administrative authority than they currently do. John used his power and resources wisely. He did not throw excess resources at someone. He gave you what he felt you needed and could use appropriately. For me, that was great because my lab grew slowly, incrementally, but in a way that I could keep up with. I started and ended working in the lab all by myself, and I love it. I truly love being at the bench and doing research. For a good number of years, through getting tenure and thereafter, I continued to work actively at the bench. I think that was great for my postdocs and technicians because I knew what was going on. When we had our ups and downs, they knew that I was going through everything with them, and I am also good at the bench. There were things that I could bring to Borrelia burgdorferi, because the genetics of that organism are not a very big field.

Harden: Hold that thought. I will come back to the details about your research if you will permit me to probe one more question about the administrative situation at this time. It was a turbulent time for staff in this period at RML. Tom Kindt [Dr. Thomas J. Kindt] became NIAID Scientific Director in 1995, and in 1997, John Swanson was removed as Chief of the Laboratory of Microbial Structure and Function. Tom Schwan was made acting lab chief. Would you walk me through this upheaval and any other changes at that time that you recall as significant?

Rosa: Yes, it was an extremely tumultuous time. I will not go into the details because I honestly do not understand them, but subsequent to her retirement, Karyl Barron [Dr. Karyl S. Barron] said that her first assignment as Deputy Scientific Director under Tom Kindt was to come out to RML and fire John Swanson. And as a new deputy director in Bethesda, that was a pretty daunting task.

Harden: Do you have any idea why they wanted to fire him?

Rosa: I think (but do not know) that it related to something that occurred in the early days of John's tenure at RML, a relationship with a junior scientist that was not acknowledged with requisite assignment to another lab. And to John's credit, whatever went on, he accepted responsibility for it. It was clearly an abrupt removal, but I think he was also at a point in his career where he believed that he had done what he could do in the Neisseria field as well as bringing RML forward. He was willing to do a few more years of research and then retire.

Laboratory of Microbial Structure and Function (LMSF) Principal Investigators: John Swanson, Seth Pincus, Patricia Rosa, and Tom Schwan, c. 1995.

Tom served as acting lab chief until Jim Musser [Dr. James M. Musser] was hired. Jim's lab was doing research at the cutting edge of bacterial pathogenesis, employing high-throughput genome sequencing, microarrays—all the very newest technical elements. Although I appreciated their value, I really liked doing things with my hands rather than with machines, so this approach was not something that I was

going to immediately embrace. I totally respect and appreciate Jim because here I was, not really a bacteriologist and certainly not an M.D., yet he fully supported me through the tenure review process. If you do not have the lab chief's full support behind you, you are not going to get tenure. Jim Musser inherited me as a tenure track candidate, clearly facilitated my getting tenure in 2000, and fully supported what I was trying to do in the lab. Although he had a style that was different from mine, and at times I was offended by it, I also could have frank conversations with him and just say, "I do not think this is the way junior people should be treated," and he would hear me, and I never suffered negatively because of that. We had a good and mutually respectful relationship, whether we did things the same way stylistically, and whether we approached science in the same way. At some point, he returned to Texas, but last summer he attended a symposium at RML to mark the retirements of four senior investigators who had been in his former lab, including me.

Jim was instrumental in creating at RML what is now called the Research Technology Branch. All of the equipment and technical know-how and personnel that he had hired to work in his own lab became part of this core group after he left. He fostered that strength at RML, which has been utilized by all of NIAID. Another element of RML that was here from the beginning and which I argued very strongly to retain when Jim Musser was lab chief, was the Media Kitchen, which is where culture media and chemical solutions are prepared for use in the labs. The Media Kitchen staff at RML have always done an excellent job. This is critical because, for example, Borrelia requires complicated growth media that are tedious to prepare and include benchmarks and standards to make sure they are of consistently high quality. That is clearly an advantage for researchers at RML, because even today, Borrelia researchers at other institutions struggle to obtain media that will adequately support the growth of Borrelia burgdorferi. We are not just looking at the DNA of the organism. We must be able to grow and manipulate it. The argument against maintaining the Media Kitchen was, "You can buy X, Y, and Z media, just like we buy plastic glassware." My argument for maintaining the Media Kitchen was, "We have people working in the Media Kitchen who have Master's degrees in science, who are established members of the Hamilton community. They are very smart people, and there is a degree of excellence here that makes it absolutely worth keeping." Also, Borrelia is excruciatingly sensitive to detergent, so the fact that the kitchen did such a fabulous job at providing us with clean, sterile glassware without any trace of detergent was critical. The media we use could not be autoclaved. It had to be filtered. There are any number of details like these that had to be addressed. The Media Kitchen is a longstanding fixture of RML that is not big and flashy, but it is a critical element of basic research that I hope continues to be supported at RML.

Harden: Now let's go into your particular research in the 1990s. From looking at your bibliography, and as a historian, not a scientist, it appears to me that you were mentally and experimentally taking Borrelia burgdorferi apart to see how it functioned, using genetic tools. You can start there and elaborate to tell me about what you were doing in the 1990s and with whom you collaborated.

Rosa: I think the thing that I brought to Borrelia and developed as a tool was molecular genetics. I loved the process of developing it, but you develop it not just for itself but to apply it and use it to ask questions. It was something that had been worked out very well in what are considered canonical bacteria—E. coli, Bacillus subtilis, Streptococcus, Staphylococcus, and other bacteria that have been studied for a good number of years. The first challenge for studying Borrelia burgdorferi, however, is that

under the best conditions, they grow slowly. So rather than doing something in the lab and looking at it the next day to see how what was tweaked might have provided an interesting result, you have to know that it takes at least one to two weeks to determine if Borrelia will grow or not. And again, it absolutely requires really good media to grow even slowly. There was a basic technique, cloning in solid media, that we had to tweak to make it applicable to Borrelia. You first disperse the bacteria you are working on, put them in solid media, and then wait for colonies to appear from a single bacterium. For E. coli, after you do that, you would typically have colonies the next morning. For Borrelia, after you do that, you wait 10 days to two weeks. Another critical consideration is that Borrelia has funny oxygen requirements. It needs some oxygen, but it does not like too much oxygen. To grow them as colonies, we had to embed the bacteria in the agarose media. You could not spread them on top, which meant that the agarose had to be warm enough to be liquid before it solidified as it cooled, but it could not be too hot or it would kill the bacteria. The technique to do this was developed by Tim Kurtti [Dr. Timothy J. Kurtti] and Ulrike Munderloh [Dr. Ulrike G. Munderloh] at the University of Minnesota.

Things like this are idiosyncratic technical details that were essential to obtaining clones of B. burgdorferi, without which we could not do genetics. My colleague and peer Kit Tilly [Dr. Kit Tilly] was the person who did the “plate and pick” aspect of Borrelia culture in my lab. Kit had been a graduate student at the University of Utah, where John Swanson knew her, and had done postdocs at Harvard, Wisconsin, and ultimately at the NIH. She came to RML as a staff fellow, but she did not become tenure track. And rather than leave and find a job somewhere else, she asked to be a technician in my lab. Although Kit was officially a technician, she was scientifically and academically my peer. She was an expert E. coli geneticist, which was valuable.

A critical component of molecular genetic studies is having a way to experimentally introduce DNA into the organism that you are studying, which is called transformation. Often you add new DNA to replace or remove something that was there before. After making the change, you can ask, "How does the organism grow now?" Or "How does it respond to this condition or that?" The only way we have been able to introduce foreign DNA into B. burgdorferi is using a technique called electroporation, which was originally applied to Borrelia by Scott Samuels [Dr. D. Scott Samuels] when he was a postdoc at RML working with Claude Garon. Electroporation drives the DNA into individual bacteria with an electrical pulse. You subject a Borrelia culture to an electrical shock in the presence of the transforming DNA. You let them recover, and then you identify the very rare variant that has survived the shock and retained the new DNA, which requires what is called a selectable marker. Typically, part of the DNA introduced by electroporation confers the ability to grow in the presence of an antibiotic, a trait that the original bacteria did not have. The transformation frequency needs to be substantially better than the frequency of spontaneous antibiotic resistance to provide a useful selection.

Harden: Wow.

Rosa: We started doing genetics in B. burgdorferi with the selectable marker that Scott Samuels had derived because the selectable markers that had been developed for E. coli did not work in Borrelia. Scott was a biochemist and familiar with a bactericidal antibiotic called coumermycin A1, which inhibits an essential enzyme called DNA gyrase. Scott knew that spontaneous resistance to coumermycin typically occurs through a discrete mutation in the gyrB gene encoding DNA gyrase. Scott treated a

culture of B. burgdorferi with coumermycin, which killed most of them and allowed him to isolate a coumermycin-resistant B. burgdorferi mutant. When he analyzed the gyrB gene from this mutant and compared it to the normal gene, he found the anticipated mutation that conferred coumermycin resistance. Scott then introduced the mutant gyrB gene into wild type B. burgdorferi by electroporation and selected for coumermycin-resistant mutants, which were recovered at a higher frequency than spontaneous mutation. This was the first description of genetic transformation of B. burgdorferi as previously mentioned.

However, we did not want to study DNA gyrase, we wanted to study other genes. To do targeted mutagenesis, a selectable marker is typically inserted into a particular gene by a process called homologous recombination, which occurs between closely related sequences on the incoming (transforming) DNA and resident cellular DNA. Scott had used this method to replace the endogenous coumermycin-sensitive gyrB gene with the almost identical coumermycin-resistant version. Although the coumermycin-resistant gyrB gene worked well as a selectable marker for transformation, it was not an efficient tool for creating mutations in other genes because the transforming DNA typically recombined with the endogenous gyrB gene rather than at the intended site. This meant that although we could now transform Borrelia by electroporation, which was a critical advance, we still had to sort through hundreds of coumermycin-resistant colonies to find the mutant we wanted.

A key advance in the genetic manipulation of B. burgdorferi, as Jim Musser acknowledged when he spoke at the symposium last summer, was the work of a very clever and hardworking postdoc named Jim Bono [Dr. James L. Bono]. Jim grew up in rural Idaho and wanted to be a cattle rancher, but that was a difficult goal to achieve if you are not born into a ranching family. Jim got a bachelor's degree in microbiology and continued in a doctoral program at Idaho State University (ISU) Pocatello, hoping that he might get back to cattle by studying something that infects them. Jim's mentor was a professor named Gene Scalarone [Dr. Gene M. Scalarone], who knew that RML was a special place for basic infectious disease research. Gene led a microbiology club at ISU and would routinely bring a group of students to RML to learn about the research people were doing. Jim Bono came up with that group, met me, and we talked. He said, "I'm going to finish my Ph.D. in Gene's lab. Do you need a postdoc?" And so he became my third postdoc.

Jim was a fastidious scientist. When we initially introduced antibiotic resistance genes from E. coli into Borrelia, they did not work as selectable markers. But Jim found that he could detect a very low level of antibiotic resistance in transformed spirochetes. His insight was in recognizing that selectable markers from E. coli might work in Borrelia if they were controlled by a Borrelia promoter, which is the engine that drives the expression of a gene. One of the hallmarks of Borrelia and every spirochete is its flagella-based motility. Jim decided to test whether the strong promoter from a flagellar gene in B. burgdorferi fused to an antibiotic-resistance gene from E. coli, would function as a selectable marker in both E.coli and Borrelia.

We had a bet book in the lab. Whenever somebody proposed something new, we would talk about it at lab meeting, and then we would have a bet. We would figure out what the stakes were—they usually involved food or drinks. The bet we had about the motility of Borrelia was whether the promoter/engine that drives expression of the flagellar gene would be continuously strong enough to confer antibiotic

resistance. This was unknown because when spirochetes are growing in liquid media, you can look at them under the microscope and see that they are wiggling/motile. However, when they reach stationary phase and stop dividing because they have used up all their food/energy source, they also stop wiggling. My concern was that colonies in solid media represent a dense congregation of bacteria. Perhaps most of the spirochetes in colonies were in stationary phase and not adequately running the promoter/engine to make flagella or to keep the antibiotic resistance cassette functional.

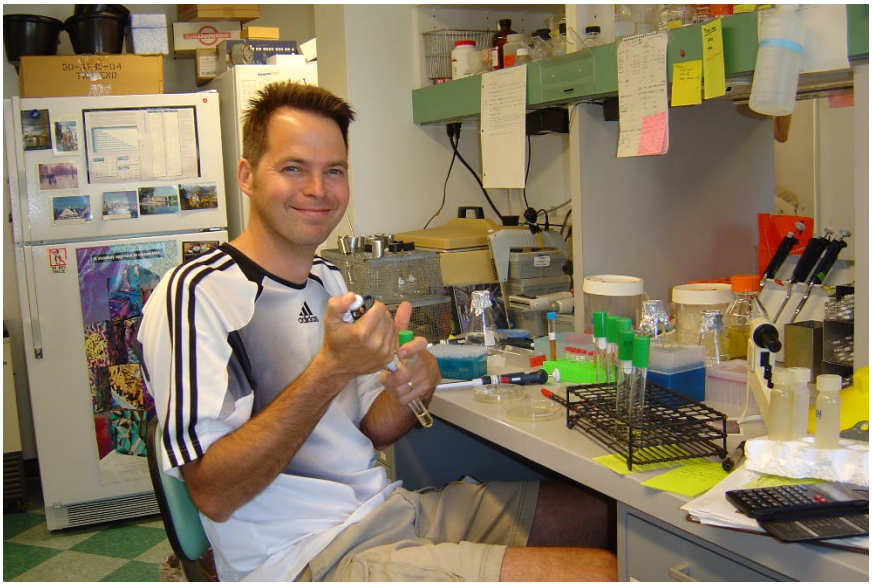
Fortunately, Jim won the bet. The hybrid antibiotic resistance cassette worked fine in both E. coli and Borrelia growing in suspension in liquid media and during colony formation solid media. That meant that we could now use a foreign antibiotic resistance gene as a selectable marker for genetic manipulation of Borrelia. We did not have to sort through the hundreds of irrelevant transformants because there was no cellular counterpart of the gene, so the only place transforming DNA could recombine was at the targeted site. The technique did not change the absolute efficiency of transformation, but it dramatically changed the ease with which we could target a gene, knock it out, and isolate the mutant, which was really key to molecular genetic investigations of Borrelia everywhere.

Lab group, 2001. Front: Abdallah Elias, Patricia Rosa, Kit Tilly, George Chaconas (on sabbatical). Rear: Phil Stewart and Jim Bono.

The next critical advance was made by Phil Stewart [Dr. Philip E. Stewart], a postdoc who subsequently became a staff scientist in the lab. We now had the ability to inactivate genes, but we did not have a genetic tool called a shuttle vector. A shuttle vector would provide a way to clone things in E. coli and then introduce the same DNA into Borrelia, where it could autonomously replicate. A shuttle vector does not have to replace anything or integrate somewhere through homologous recombination, you just need to introduce the plasmid DNA into the bacteria and it is maintained. We realized early on that the genes

that allow E. coli plasmids to persist in E. coli did not confer this trait in Borrelia. One of the unusual molecular features of Borrelia is the segmented organization of its genomic DNA. Most bacteria have a single circular chromosome, whereas Borrelia has a linear chromosome and more than 20 linear and circular plasmids. Alan Barbour described this unique feature of Borrelia when he was working with Claude Garon at RML in the mid-1980s. Phil focused on the smallest plasmid and asked, "If I fuse this Borrelia plasmid with the antibiotic resistance cassette and an E. coli plasmid, will it autonomously replicate in both E. coli and Borrelia?" The answer was yes!

Phil went on to figure out exactly what segment of plasmid DNA is required for autonomous replication in Borrelia. He took a careful look at the nucleotide sequences of the 20+ plasmids in Borrelia and identified a region of DNA that was conserved, but slightly different in each one. Phil proposed that the ability of so many co-existing plasmids to replicate in Borrelia relied upon similar, but unique DNA sequences on each plasmid. Another feature of plasmid biology is called incompatibility. Two distinct plasmids that utilize exactly the same machinery for autonomous replication will not coexist. They either fuse to become a single plasmid or one of the plasmids is lost from the cell, which is called displacement. Given this feature of plasmid replication, how does Borrelia maintain 20+ different pieces of genomic DNA, all happily coexisting? Phil also demonstrated that individual Borrelia plasmids were incompatible with a shuttle vector carrying their cognate replication region but could coexist with shuttle vectors with replication regions derived from other Borrelia plasmids.



Dr. Phil Stewart in lab, 2004.

Another unusual feature of Borrelia plasmids is that they are present at approximately the same copy number as the chromosome. In other bacteria, there are one or two copies of the chromosome per cell, and the plasmid copy number can range widely. The chromosome is the essential genetic element in most bacteria, whereas plasmids are typically accessory pieces of DNA that confer a selective advantage under particular conditions. However, Borrelia plasmids carry a number of essential "housekeeping" genes that are typically encoded on the chromosome in other bacteria. The first postdoc in my lab, Neil Margolis [Dr. Neil Margolis], showed that two essential nucleotide biosynthesis genes were uniquely present on a plasmid in Borrelia and not on the chromosome. In addition to developing the antibiotic

resistance cassette, Jim Bono showed that there are other genes on the same plasmid with housekeeping functions. Despite having so many pieces of DNA, Borrelia has a very small genome and limited metabolic capability. Most of what the Lyme disease spirochete needs to survive has to be imported from either the vertebrate host or the tick vector. B. burgdorferi colonizes the midgut of the tick vector and only replicates when blood is present during and immediately following tick feeding.

Yet another striking feature of Borrelia is the spirochete's ability to survive indefinitely without replicating in an unfed tick. Ecologists and vector biologists have demonstrated that unfed infected Ixodes ticks can survive for a long time in nature —probably three to four years, and still transmit B. burgdorferi when they next feed. These bacteria are like symbionts: they are surviving, they are not hurting the tick, and they are dependent on the tick blood meal for replication and transmission. This ability of the Lyme disease spirochete to survive for an extended period of time in a nutrient-limited environment is not replicated in the lab. When we propagate B. burgdorferi in growth media, the bacteria replicate to a certain level and stop dividing, and after about a month, they are all dead. There's obviously a discrepancy between how B. burgdorferi grows in culture medium versus in the tick vector. The Lyme disease spirochete Is only transiently present in the blood of infected vertebrate hosts but can disseminate and persist for years in various host tissues. The absence of spirochetes in the blood and low numbers in tissues stems from the acquired immune response of the host rather than nutrient limitation.

To get back to the genome, as mentioned previously, B. burgdorferi has several plasmids that contain essential genes that are necessary for spirochete growth and survival in vivo. There is also an intriguing set of plasmids that was investigated by another clever postdoc, Brian Stevenson [Dr. Brian Stevenson], who is now a professor at the University of Kentucky. Brian was the second postdoc in my lab, and at that time (pre-genome sequence) we knew that B. burgdorferi contained an abundant species of circular DNA that was ~32 kilobases (kb) long. What Brian was able to demonstrate, working with Sherwood Casjens [Dr. Sherwood R. Casjens] at the University of Utah, was that this piece of the spirochete genome was actually composed of 10 distinct circular plasmids that were all 32kb in size, but with slightly different plasmid replication region that allowed them to co-exist.

One of the things we did in collaboration with Tom Schwan addressed the difference between B. burgdorferi grown in the lab versus what happens in vivo. The spirochetes grow well in culture media and they typically make a lot of a certain plasmid-encoded outer surface protein called OspA. However, in the natural infectious cycle between ticks and mice, the OspA protein is only made by spirochetes in the midgut of an unfed tick when they are not replicating. Somehow the signals for "make this and do not make that" are mixed up in culture medium. Tom showed that if you shift the spirochetes in culture to a cooler temperature and then warm them up, they will make new proteins that had not been seen before. These temperature-induced proteins are also made by rapidly growing spirochetes in feeding ticks, before and during transmission to a mammal. We were able to show was that one of these temperature-induced proteins, OspC, is absolutely critical for survival during the initial stage of infection in the host. We still do not know what essential function OspC fulfills immediately after transmission, but it is a plasmid-encoded outer surface protein that is not typically made when spirochetes are growing in culture medium. Brian studied a number of additional proteins that were induced in temperature-shifted

cultures and during tick feeding in infected ticks, many of which were encoded by different 32 kilobase plasmids.

We had a long-standing collaboration with a colleague at the University of Utah, Sherwood Casjens. Sherwood had studied bacteriophage, which are viruses that infect bacteria. Bacteriophages have different ways of existing. Once inside a bacterium, they can become a prophage, with their DNA incorporated into the chromosome or as an autonomously replicating plasmid. In response to particular signals, prophage can convert to a lytic phase, replicate to high levels, lyse their bacterial host and infect new bacteria.

Sherwood, being a phage guy, looked at the 32 kb plasmids and deduced that they resembled hetero-immune prophage, complete with a classically organized phage genome. So now jump forward many years, through a lot of studies by talented members of my lab and many other labs in the field, to the last postdoc in my lab, Jenny Wachter [Dr. Jenny Wachter], who is now at the University of Saskatchewan, Saskatoon. We have known for quite a while that there is a master regulator of temperature-induced protein synthesis in B. burgdorferi in culture and during tick feeding. We have also known for a long time that spirochetes cannot make too much of that master regulator or they die, but we did not know why. Jenny has been able to show that over-production of the master regulator is lethal for spirochetes because, in addition to inducing proteins required for survival in the host, it stimulates replication of prophage plasmids, presumably resulting in a lytic phase. Jenny was able to demonstrate that this naturally occurs in the infected tick midgut during tick feeding and transmission, but then the master regulator gets shut off. Induction of phage while preparing for transmission would be beneficial for B. burgdorferi in nature because phage can mediate DNA exchange among spirochetes. In Lyme-endemic regions, naturally infected ticks usually carry multiple strains of Borrelia burgdorferi and vertebrate hosts are repeatedly fed upon by infected ticks. OspC defines the serotype of B. burgdorferi, and there are ~20 different OspC types. This means that spirochetes cannot reinfect a host with the same OspC type, but they can reinfect a host with a different OspC type. Induction of phage and resulting DNA exchange among spirochetes in the tick midgut during feeding would allow them to shuffle OspC genes and facilitate transmission to previously infected hosts. I would like to add that I have mentioned only a few of the gifted scientists who worked in my lab over the years. They all made significant contributions to my research program and to the Borrelia field, and I could have described their findings with equal pride and enthusiasm.

Harden: From the taxpayer's point of view, how is your work going to help control Lyme disease? I am sure that you have been asked this before because you have to justify what you are doing in order to be supported for the research. Could you speak a little to that question?

Rosa: There are two ways our work helps to control Lyme disease. First, let me talk about vaccines, and then I will go into a broader picture of why what we do matters. With respect to vaccines, our work matters very directly in terms of how current and future vaccines work. I have talked about OspC, but the original vaccine for Lyme disease was based on OspA, which is what the spirochete makes when it is growing in culture medium in the lab, and in the midgut of unfed ticks, but not when it is in an infected host. There is another vaccine that is licensed for dogs, and I think there is a similar one on the cusp of being licensed for humans, that is directed against OspC. As described above, we know that antibodies

that recognize OspC can prevent infection. The effective OspC vaccine that's being used in dogs was developed by Rich Marconi's [Dr. Richard T. Marconi] lab at Virginia Commonwealth University. What they have been able to show is the exact region of OspC that an antibody recognizes, which varies between different OspC types. They have made a chimeric protein that includes this serotype-defining region from multiple OspC types. This vaccine gives good protection in dogs and demonstrates that OspC is clearly an excellent vaccine target. Other groups have developed other OspC-based vaccines, potentially for use in humans or rodent reservoir hosts. So the simple reason for why studying OspC matters is because it is a very good vaccine target for Lyme disease, and the more we can understand about it, the better.

With respect to the bigger picture of why doing genetics research on B. burgdorferi is worthwhile, there are so many things central to infection and transmission of the Lyme disease spirochete that are not understood. We still do not know much about phage, but I am confident that in the not-too-distant future, there will be major advance in the Borrelia field that involves phage. We know from our research that if we unleash phage replication, the Borrelia spirochetes are dead, which may provide ways of controlling the spirochete population.

But to be honest, I am not driven by potential utility. I am just fascinated by a question and a puzzle. I do not apologize for it, because I believe that we do not know what will be the next big thing. Certainly, there have to be people willing and able to do applied research in order to show how it matters. I understand that NIH administrators obviously have to satisfy taxpayers that we are not looking at things merely for the whimsy of looking at things. But understanding at a basic research level how something works can have unpredictable positive outcomes and ramifications. Some people dislike working on things without a foreseeable application, but others (like me) go crazy if the path ahead is clearly defined. I learned during my first rotation in grad school was that I was not happy repeating the same technique every day in the lab.

When I first came to RML, I enjoyed identifying a unique sequence in B. burgdorferi and developing a good PCR diagnostic test from it. Then, when I used it to determine whether a particular mouse was infected or not, it became obvious that very few spirochetes were present in infected samples. Regardless of the specificity of the assay, PCR was not a good diagnostic tool for human or mouse infections with B. burgdorferi. I also realized that doing experiments that yield yes/no answers, even if you can answer complex biological questions with them, doesn't satisfy me. Fortunately, other people love to do that. Part of the reason Kit Tilly and I worked so well together was that we liked to do different types of experiments. She loved E. coli genetics. She enjoyed looking at a plate every day and picking colonies while she thought about the results of the previous experiment or designed the next. I like to do different experiments that may or may not work, think about what I am doing and how it might be improved or done differently. They are both good approaches, and it takes people with compatible and complementary skills to move basic research forward.

Dr. Kit Tilly in lab, 2004.

Harden: You have articulated here how different skills are needed and how different kinds of people are needed to work on basic and applied research. I am always interested in what it is that motivates somebody, what kind of psychological feedback keeps someone in the lab.

I want to move along here first with a small question related to how RML's geographic footprint expanded after the terrorist attacks of 9/11. A lot of research became focused on ways to counter bioterrorism, but your lab stayed focused on Borrelia, as I understand it. Do you want to make any comments about the changes that were occurring at RML during this time?

Rosa: I think they were appropriate, in terms of RML and its capabilities for NIAID. What I had to compare them with was the situation when I first got to RML. There were protests by PETA [People for the Ethical Treatment of Animals] because some RML researchers were using mink in research studies. Marshall [Dr. Marshall E. Bloom] probably mentioned that to you because he studied a virus that infects mink. In addition, there is a non-human primate colony at RML. I am grateful for not having to work on non-human primates, but I understand when and why they need to be used. And then there is all the COVID-19 [Coronavirus Disease-2019] work that happened at RML. Marshall has been fabulous at interfacing with the community. He has been known for a long time in Hamilton and he enjoys interacting with people in the community. Not my cup of tea and thank God that Marshall was there and willing to do it. After the terrorist attacks of 9/11, there were multiple levels of security. The 1995 bombing of the Oklahoma City federal building had preceded the 9/11 attacks, so there was already concern about the level of physical security at a federal facility. Before all this, my daughters could sit on the lawn outside my lab window, and I could open it and talk to them. Now none of the windows at the lab can be opened. There is fencing all around the campus and you need a badge to enter or somebody to escort you.

For Borrelia, we have to stand on our head to make it grow in the lab under optimal conditions. It does not present a threat to public safety, nor do the Ixodes ticks that we maintain in the lab to study transmission, which don't exist in Montana. It is too cold and too dry, so even if Ixodes ticks escaped from their humidified container, they would not survive. The history of RML goes back to the identification of Rickettsia rickettsii as the causative agent of Rocky Mountain spotted fever (RMSF). The residents of the Bitterroot Valley were aware that wood ticks on the west side of the Bitterroot River could potentially transmit RMSF, but those on the east side could not. When RML was built by the state of Montana in 1928, Hamilton residents worried that infected ticks might escape and introduce RMSF on the east side of the river. The specific concerns of people in Hamilton have changed, but it is a community, and RML is now a federal research facility. On one hand, many members of the Hamilton community recognize and appreciate how much RML personnel and federal money have provided to Hamilton. The reaction of other people is, "Oh my God, there's the lab." They may be happy to take advantage of the benefits of a federal facility, but they resent the fact that it is here.

I have complained about the never-ending construction at RML and the impact on the surrounding neighborhood, but there are valid reasons for it. If NIAID's program is growing in a way that can be best addressed at RML rather than in Bethesda, then it is appropriate, and NIAID pays attention to our complaints as much as possible. For example, landscaping at RML has finally received the attention that it deserves. You cannot just construct new buildings and leave the landscape looking like a gravel pit. We are part of a neighborhood community and we work in this environment. The Bethesda campus looks nice, so why shouldn't the RML campus also look nice? After much complaining by RML staff, NIAID enlisted landscape professionals to design and upgrading plantings.

Harden: In the 2000s you served on all sorts of committees. In addition to the expected ones related to Lyme disease and spirochetes, you served on a CDC search committee for a bacterial zoonosis branch chief. You served on NIAID intramural Promotion and Tenure (P&T) committee, a peer review steering committee for the American Heart Association, and more. How did you manage all these outside activities with your research? And please tell me specifically about how your work interfaced with the American Heart Association (AHA), which seems strange to me, but probably it has a very good connection.

Rosa: The connection between Lyme disease and heart disease is a good but less frequent outcome. Borrelia burgdorferi infection is most known for Lyme arthritis, but it can also cause Lyme carditis. The American Heart Association has a broad picture of recognizing these different aspects. I was first on an AHA microbiology committee, and then I eventually became chair. AHA primarily funded early career researchers who did not have NIH grants. They would apply to either the Arthritis Foundation or the American Heart Association for support. Both organizations were willing to support a broad swath of research that explored how Lyme disease affected the joints or the heart. The AHA microbiology committee reviewed grants that not only focused on Borrelia but also included a number of other bacteria that affected the heart.

I think I ended up chairing both the AHA grant review committee and the NIAID promotion and tenure committee because of what I could contribute as a leader rather than as a microbiologist. These committees took a lot of time, but I felt capable of fulfilling my role, and the committees were highly

worthwhile and fulfilling. One aside, with respect to promotion and tenure, certainly at NIAID, and possibly NIH-wide, is that there has been a substantial change in how the tenure-track process goes forward. The Institute needs to provide adequate resources and treat all tenure-track candidates comparably. However, these early career scientists have to be allowed to show what they are capable of on their own. It seems to me that sometimes there is too much effort to assist, to make sure they get the resources and the support they need, that candidates end up spending too much time addressing mentoring committees rather than conducting research.

Harden: During the early 2000s, you also started collecting awards. In 2001, you were elected as a fellow in the American Academy of Microbiology. In 2007, you received an NIH Merit Award and in 2012, another merit award from NIAID. And in 2009, you received an NIAID's Outstanding Mentor Award. Would you tell me a few details about the two merit awards from NIH and NIAID, and then more about the Outstanding Mentor Award?

Rosa: I will start by giving you a cynical point of view, as I do not put much weight in Merit awards. I think they just happen. Do I think I was deserving? Yes. I think we made significant advances to the field in terms of genetics. We developed techniques that were not possible modes of investigation before then. How or why one gets a Merit award, I honestly have no idea. I think it is partly politics. I am not saying that they are not deserved, but there are probably plenty of deserving people who do not get Merit awards. Jim Musser is the reason I was elected to the Academy of Microbiology. He nominated me and supported my candidacy, and I am grateful to him.

The NIAID Outstanding Mentor Award is the one that means the most to me. I think I know who recommended me for it, but at the time I had no clue. When the selection committee was reviewing nominations, they did not know the identity of the nominee, they just read about the person's capabilities. Can you imagine how challenging it would be for a visiting fellow to arrive in Hamilton in the middle of winter? Someone who had never before been out of their home country, had always lived with their family, and didn't know how to drive. They might speak English, but not extremely well, and they would not have a place to live, a credit card, a record as a tenant or a bank account. I believed that my job was to actively assist newly arrived fellows in my lab, help them find a place to live, establish a bank account, and provide transport as needed, because there is no local public transport. So Paul and I would initially welcome fellows into our home and help them get settled in the community. Our daughters were both obliging and complaining about the number of strangers they had to have breakfast with as they grew up. But how could you expect somebody to begin their career at RML without such assistance? Eventually, they had their own apartment, learned to drive, and become independent people, but that was not possible when they arrived. At some point, we were told that we could not help trainees find housing, and we absolutely could not house them ourselves. I was offended because I felt that this was inhumane. I wrote to Michael Gottesman [Dr. Michael M. Gottesman, NIH Deputy Director for Intramural Research] to express my opinion, but it made no difference. This had become an NIH policy.

Harden: In 2005, a Laboratory of Zoonotic Pathogens (LZP) was created with Tom Schwan as Lab chief. You, Frank Gherardini [Dr. Frank C. Gherardini], Joe Hinnebusch [Dr. B. Joseph Hinnebusch], and Tom—three tick people and one flea person, as Tom told me—were the tenured investigators. Is there anything about your research in this new lab that might have differed from what you were doing in the past?

Rosa: During this time, RML was growing and changing. First, there was a remodel, in the 2000s, when Jim Musser was around. This remodel had to do with bringing RML up to federal fire and earthquake standards, because the existing old brick buildings did not meet federal standards. The question was, do we keep these buildings or do we replace them? If retained, they had to be modified to meet federal fire and earthquake standards. The decision was made to keep the original buildings, so our labs moved around as they sequentially remodeled buildings. I think my lab and office moved three times, and the last time was my choice, because I wanted a lab on the corner with a southern exposure, and I was senior enough to ask for it. That was the space previously occupied by Jim Musser's lab. By then, Tom was lab chief and could have claimed that lab, but he was totally supportive of my lab moving there.

The labs kept changing names. There was the Laboratory of Microbial Structure and Function under John Swanson, which became the Laboratory of Human Bacterial Pathogenesis (LHBP) under Jim Musser. There were a couple of junior investigators who stayed in LHBP, and then LZP was formed under Tom Schwan. Harlan Caldwell left, so the Laboratory of Intracellular Parasites (LICP) merged with the remnants of LHBP and became the Laboratory of Bacteriology (LB) under Frank DeLeo. And then Tom retired and I became lab chief for LZP in 2014. It was clear to me that there was not enough scientific critical mass in LZP. To sustain the lab, we needed more PIs, but there was a hiring freeze and NIAID was not recruiting any tenure-track investigators. Although administratively separate, we combined our weekly research presentations with LB, and the two labs officially merged at the end of 2017, with Frank DeLeo [Dr. Frank R. DeLeo], as lab chief.

To be honest, can I be a lab chief? Yes. Did I enjoy being a lab chief? No. There is a lot of responsibility without a lot of authority. Unless you somehow relish the title, for me it was that much more time spent in front of a computer, rather than thinking about science.

Kit Tilly's crew in Laboratory of Zoonotic Pathogens (Patricia Rosa, Lab Chief), 2014. Seated L->R: Dorothee Grimm, Lisa Fazzino, Kit Tilly, Iris Olivas, Chenyi Chu, Irene Kasumba. Standing L->R: Patricia Rosa, Claire Checroun, Jennifer Errett, Abdallah Elias, and Phil Stewart.

Harden: Your comments have raised questions for me. You said that you were asked to serve as lab chief because you were a senior woman. Is that a broader commentary, that, in other words, NIAID had reached a point at which they were aware, A, that you were qualified, and B, that they needed to promote women, and so they did. Would you provide some additional detail here? You became lab chief in 2014. Did you retire before Frank DeLeo became lab chief or afterwards?

Rosa: I was chief of LZP from January 2014 through 2017, when it merged with LB. Kathy Zoon [Dr. Kathryn C. Zoon, NIAID Scientific Director] asked me to become Lab chief when Tom Schwan announced his retirement. She and Karyl Barron [Dr. Karyl Barron, NIAID Deputy Scientific Director] said, "Look, Patti, you do not want to do this, but we need a woman to be lab chief." At that point, LZP was in a holding pattern and NIAID was not willing to expand it. In 2016, when Steve [Dr. Steven M. Holland] took over as NIAID Scientific Director, it was clear that there was no immediate plan for recruiting either tenure-track people or a new lab chief for LZP. I had already decided that LZP needed to merge with LB in order to have a broader group of people to interact with. So LZP was folded into LB at the end of 2017, before I went on sabbatical in 2018.

I had a long-standing interest in going on sabbatical. Steve Holland was supportive of it, although, to be perfectly honest, nobody ever went on sabbatical, at least not from RML. So they had no clue, administratively, how to make it happen. I think I was technically an RML staff member, but my work station happened to be in New Haven rather than Hamilton. I was on sabbatical in Christine Jacobs-Wagner's [Dr. Christine Jacobs-Wagner] lab at Yale from 2018-2019. It was as fabulous as I had anticipated in being able to work in the lab on a topic that I was interested in—the DNA of Borrelia. Christine's lab was pretty new to the Borrelia field, but they were really good basic researchers and extraordinary microscopists, with the capability to directly visualize the DNA in Borrelia. It was a mutually fruitful collaboration. They had great software capabilities, so they could capture a large number of microscopic images and then combine them to generate an overview of what was going on. They had the ability to fluorescently tag individual DNA molecules. Their ability to genetically manipulate B. burgdorferi was not quite there initially, primarily because they did not have optimal growth media. I was able to help them get up to speed in very basic ways and then apply their really sophisticated tools to another Borrelia project that I brought from RML. I worked with an M.D./Ph.D. student named Zach Kloos [Zachary Kloos] and a postdoctoral fellow named Nick Takacs [Dr. Constantin (Nick) Takacs], who were both talented, enthusiastic and extremely helpful colleagues. It was fun to go to the lab and spend most of each day interacting with the postdocs and grad students. Sabbatical was wonderful. Christine Jacobs Wagner and her lab have moved to Stanford, where they continue to investigate the mechanics of plasmid maintenance in Borrelia. I hope they can someday visualize the prophage as they switch from normal replication to lytic phase.

Harden: Did your husband go with you?

Rosa: No. As I said, he had moved around a lot as a kid, and I think he did not want to be a tag-along spouse. He also had a grim mental picture of New Haven: "like Brussels, dark and gray." So I typically returned to Hamilton, or he came to Connecticut, about once a month during my sabbatical. Our daughter Justine and her family live in Manhattan, so I would occasionally take the train into New York

on the weekends, or they would come up to Connecticut. Christine's lab was on Yale's West campus, and I lived in a little town on the coast called Milford. I could drive to the lab on back streets and my apartment building was close to the train station. It gave me confidence to realize I could do this on my own. It also made me realize how much I enjoyed sharing life with my husband when we were together.

Harden: Your CV does not have absolutely clear retirement date for you. When did you retire fully? When did your husband retire, if he did, and what are you doing now?

Rosa: I retired fully at the end of April of 2023. My husband has not yet retired. He has submitted his last paper and hopefully will retire within the next six months.

What am I doing now? I am trying to wrap up a couple of manuscripts with Jenny Wachter and Nick Takacs on projects that I worked on at Yale and RML. One of the things that I did not satisfactorily complete before I retired was the long-term storage of the genetic constructs and mutant Borrelia strains we had generated at RML over the years. This is somewhat similar to what happened to the tick collection at RML long ago. I do not know if Tom mentioned it to you when you interviewed him.

Harden: I know about the tick collection from when it began in the early 20th century from my work on the history of Rocky Mountain spotted fever.

Rosa: What we had generated at RML was an extraordinary resource for the Borrelia field. I had freezers full of mutant B. burgdorferi strains. I could document the numerous requests I had received from other investigators for various reagents. I began a conversation with NIAID administration and tech transfer well in advance of retirement, but they never figured out what to do with our frozen Borrelia strains. I made sure that Jenny Wachter had everything she needed, that Nick Takacs had everything that he needed, and that colleagues who knew that I was retiring had what they needed from our collection. Phil Stewart, who had moved to Marshall Bloom's lab, retained a basic collection. Biosafety needed to certify that there were no more pathogens in any of my freezers before I retired. So, other than what went to Phil, and to a couple of tenure-track investigators in LB who are working with Borrelia, I autoclaved everything else.

One of my goals is to learn Latin, because I love language and order. Other than that, I walk, I swim, I read, I like to garden. I am a rather domestic person. I like my environment. I like taking care of it. There are so many things to do. We live not far from Hamilton on two acres, so we have a big yard. This means that in the summertime you are never completely done with all the tasks involved in taking care of it. In the winter, there are the usual challenges—the ground source heat pump may not be working properly, or the window coverings are inadequate, we need wood for the wood stove so that the pipes do not freeze. I love music. We have a condo in Missoula. We usually go there every weekend just to do things that are available in Missoula. It sounds lazy. It is lazy, but I love it. I also enjoy traveling and spending time with our daughters and their families. It's very good to interact with Justine and Elise as adult women, and to watch their children grow up.

Harden: These are all the questions I have. Is there anything else you would like to get on the record before we stop?

Rosa: The one overall thing I could add relates to being a woman in science.

Harden: Yes. Please go on.

Rosa: Even with ample self-doubt along the way, I knew that I was not going to be satisfied until I was doing my own thing. That is not true for everyone. Women (and men) should not feel compelled to strive to be an independent investigator if they would rather play a different role. But if being an independent investigator is what you want, then you can make it happen. In addition, do not let slights—even if they were intentional slights—hold you back. I don't think that you should ignore things that are really bad, but you do not need to go out of your way to find trouble. There is not enough time or energy for trivial matters when you could have fun thinking about science.

Harden: Thank you. And I thank you for a fine oral history.