Stanley Falkow

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The Fortunate Professor

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Abstract

My professional life can be summarized by a quote from the Talmud.

> Much have I learned from my teachers,
>
> More from my colleagues,
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> But most from my students.

It is the fortunate professor who learns from the student.

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INTRODUCTION

I am honored to be asked to contribute the Prefatory chapter for Volume 62 of the *Annual Review of Microbiology*. The mandate was to provide a retrospective view of my career in microbiology, and I was encouraged to include personal reminiscences and events that were significant to me. This is not an easy task under any circumstance. In my case, it took on added meaning because three years ago I was diagnosed with a bone marrow disease, which led me to begin to bring my career to an end. I feel quite well and my disease, like a number of microbial diseases I studied in my career, has an imprecise natural history. The state of my health was known to some of the Committee Members when I was invited to submit this article. I thought, "Well, I guess they wanted to invite me before it's too late."

I would like to take this opportunity to share some of the things I learned in my professional life and to outline the help and guidance I received from so many people along the way. During the four decades I worked as an active scientist, I helped train 35 graduate students and 61 postdoctoral students and infectious diseases fellows, and I collaborated with and published scientific reports with 75 other scientists. I dedicate this article to them, especially my students. I must apologize; I could not possibly mention them all or thank them all sufficiently in the space provided for this short memoir.

MY DECISION TO BECOME A BACTERIOLOGIST

My father, Jacob Falkowitzki (?), was born in Kiev before World War I and immigrated to Albany, New York; my mother, Mollie Gingold, was born in the United States after her family arrived from Bialystock, Poland. I was born in 1934, at the peak of the Great Depression. My first language was Yiddish, and my early years were spent in a noisy, colorful environment of tenement row houses filled with a mélange of languages, smells, and customs.

In 1943 my family moved to Newport, Rhode Island. I was taken from an intense urban neighborhood of Jews, Italians, and Poles into a conservative New England seaside community. I attended a public school and was a terrible student by both objective and subjective criteria, but I loved to read, although I didn't want to read what was assigned to me in school. Somehow I discovered the public library and happened on a book called *Microbe Hunters* by Paul de Kruif. De Kruif describes in colorful detail the microbe hunters Louis Pasteur, Robert Koch, Paul Ehrlich, Elie Metchnikoff, and others, who showed that microbes could cause disease. He described their work to understand the human systems that oppose infection and their search for cures. These microbe hunters became, and remain, my heroes. Their search to understand microbes was to me the most extraordinary adventure that I could imagine. It still is.

Thus, at age 11, I decided to become a bacteriologist. My first view of bacteria was in spoiled milk, barely discernable in my Gilbert Company Hall of Science microscope, but no doubt about it, here were Antony van Leeuwenhoek's tumbling animalcules. Protozoa in a grass infusoria were even more wondrous. I wish I could report that these revelations had a major impact on my endeavors as a student. They did not. I continued my record of failure in mathematics,

and my science grades in chemistry and biology were average, at best. Fortunately, I was put on the right path through the intervention of Lottie Brindle, a remarkable English teacher, who told me, among other things, "If you stop being such a wise guy and listen, you just might make something of yourself." I cannot say that I underwent a complete transformation, but I became a better student.

THE UNIVERSITY OF MAINE (1951–1955)

I left Newport to attend the University of Maine in 1951. After years of declaring that I wanted to be a bacteriologist, I suddenly realized I had fallen in love with a fantasy I had conjured up from a description in a book. At Maine I finally applied myself and became immersed in serious laboratory exercises in chemistry and biology. Toward the end of my freshman year, I wrote a letter to the Newport Hospital offering my services during my summer break with the stated hope to be taught something about bacteriology. They replied that they would be happy to employ me, without pay, to work in the clinical laboratory. My duties would include helping the laboratory technicians, assisting at autopsies and helping the hospital bacteriologist, Alice Schaeffer Sauzette, who had developed a well-known endospore stain (22). In the summers that followed, Alice showed me how to identify the microbes appearing on the plates of growth media on which we spread bits of effluvia emanating from within or on the human body during health and disease. I gram-stained all of it and looked at the slides using a real microscope.

This experience exceeded my childhood fantasy! During that first summer, I saw most of the common infectious diseases of that time and often isolated the microbes from the sick patients. If there were a case of something unusual like diphtheria, meningitis, or whooping cough, I would arrange with a physician to be present when he examined that individual, and sadly, sometimes I saw the patient again on the autopsy table. Many of the physicians at the hospital knew of my interests and would talk to me about infectious disease epidemics of the past and reminisce about the days before antibiotics. I was fortunate to have been exposed to the discipline of medical microbiology and infectious disease so early in my career; it helped me understand host-pathogen interactions before I even knew that this was the path of research I would follow.

In my junior year I finally took a university course in general bacteriology. There was only one other bacteriology major, Peter Pattee, who subsequently became a distinguished professor at Iowa State University. I showed off my skill at streaking plates and performing the routine chores of culturing and identifying bacteria. I am happy to report that my professors in the bacteriology department, particularly my advisor, E. R. Hitchner, took my self-importance and misplaced pride in stride and then firmly, but gently, pointed out my ignorance.

My undergraduate bacteriology training at Maine included fermentation mechanisms, where we prepared sauerkraut and studied how each group of microbes interacted and sequentially cooperated to transform a crock of alternating layers of shredded cabbage, salt, and water into a wonderful accompaniment for bratwursts. I learned dairy bacteriology. I soaked up virology, parasitology, and, at last, a formal course in medical bacteriology. Hitchner presented soil microbiology lectures with all the reverence of a parson preaching to his congregation. He made me see that microorganisms live not as pure cultures, but as members of complex communities that often depended on one another for their survival. To get the baccalaureate degree in bacteriology, it was also necessary to do a research project and to write a report of the findings. I studied the L-forms of bacteria and this little study eventually became my first scientific paper (5).

GRADUATE SCHOOL (1955–1960)

I started graduate school at the University of Michigan in the fall of 1955. Three events followed that had a major impact on my life. First, I met Allan Campbell, a new assistant

professor. I was Allan's teaching assistant, and he gave me a copy of the new book, *The Microbial World*, written by Roger Y. Stanier, Michael Douderoff, and Edward A. Adelberg, which was to be the foundation of his course. He also introduced me to bacterial genetics.

Second, I began to suffer panic attacks. Today, this biochemical anomaly can be treated with a variety of drugs and behavior modification; in 1955, there was nothing available. I've written about this before (7), and there is no doubt it influenced my personal and professional life immensely. The immediate effect was to stop my graduate education. I returned to my job at Newport Hospital. In the evenings I did research, which led to the publication of two papers describing biochemical tests for distinguishing between different species of enteric bacteria, one of which, I'm told, is still used occasionally (6).

Third, these papers put me in contact with C. A. Stuart at Brown University. I asked him how bacteria became pathogenic and how the enteric species had evolved. Rather than answer, he introduced me to Herman Chase, a mouse geneticist, who handed me a copy of a new book, *The Chemical Basis of Heredity* (20). Chase suggested I read as much as I could and come back weekly and we'd discuss any questions I had. I belatedly learned about the discovery of DNA and had the privilege of reading papers by Seymour Benzer, Francois Jacob, and other greats of that era.

Thanks to Chase and Stuart, I was given a graduate fellowship and started graduate school at Brown in the fall of 1957 working in "Doc" Stuart's laboratory. Seymour Lederberg joined the Brown faculty that year. Seymour had worked with Salvatore Luria and planned to duplicate the storied Cold Spring Harbor phage course for Brown biology students. I was Seymour's teaching assistant, and he tutored me as I performed each of the experiments prior to lab class. It was a marvelous opportunity that served me well thereafter.

Then, and today, a first-year graduate student is introduced to the intricacies and uncertainties of scientific papers and is taught to understand the strength of the work that is reported and its possible flaws. In addition, I took courses in genetics taught by Chase. I remember especially Elizabeth LeDuc's cell biology lectures seemed like poetry. All my professors were scholars who asked us innocent questions that had no answers. They led my classmates and me to the cutting edge of thinking about the biology of that era. They posed questions I had never contemplated, and now forced to consider them, I was humbled and yet challenged. Graduate school was one of the happiest times of my professional life.

WALTER REED (1960–1967)

Doc Stuart gave me a paper to read written by Louis S. Baron on the transfer of *Escherichia coli* genes to *Salmonella typhi*. Norton Zinder published similar results using *E. coli* and *Salmonella typhimurium*. I duplicated Baron and Zinder's results and spent most of the summer of 1959 reading everything I could lay my hands on about *E. coli* genetics and performing genetic crosses between different *Salmonella* and *E. coli* Hfr donors. The time had come to do my own genetic experiments and not just read about the work of others. Stuart was near retirement and had severe heart disease so he sent me in September 1960 to perform my thesis research in Lou Baron's laboratory at Walter Reed Army Institute of Research (WRAIR).

Lou was a wonderful man and a gifted bacterial geneticist. He asked me prior to my arrival to work on a naturally occurring *S. typhi* clinical isolate that was notable for its ability to ferment lactose and transfer the *lac*$^+$ genotype at high frequency to other bacteria. My experiments indicated that this strain was nature's version of the F-*lac* episome, which had recently been described for *E. coli* K-12 by Ed Adelberg and Francois Jacob. Moreover, the lactose gene was transferred from the *S. typhi* donor to many different kinds of lactose-negative bacteria. To my astonishment, the experiment even worked for *Serratia marcescens* and *Vibrio cholerae*.

I performed an experiment violating the conventional wisdom that gene transfer only

occurred between closely related bacteria. Like every other biology student of that time, I knew about the 1958 Meselson-Stahl experiment on the nature of DNA replication based on showing that DNA of different density, including differences in base composition, could be separated in concentrated solutions of cesium chloride (CsCl). *S. marcescens* had a G+C content of 58%, while *E. coli*, *Salmonella typhi*, and *Shigella flexneri* DNA were all 50%–52% G+C. In a CsCl gradient, molecules with an overall composition of 50% would band at a totally distinct location compared with a 58% G+C molecule. I wondered if the DNA from a culture of *S. marcescens* carrying the F-*lac* genetic factor might show two distinct bands in the CsCl gradient, one corresponding to the chromosome of *Serratia* and the other to a purported extrachromosomal element like the F_0-*lac* factor transferred from the *S. typhi* donor. It was a simple experiment. That is, it was simple if I could make the DNA and if I had a Model E ultracentrifuge and knew how to use it. Within a month, Baron and Sam Formal, who has remained a wonderful mentor to me, arranged an American Society for Microbiology (ASM) travel fellowship and the Army's blessing to work with Julius Marmur at Brandeis University to test this idea.

I spent two weeks at Brandeis under Marmur's guidance. I learned how to purify bacterial DNA. It sounds so trivial now, but in 1960 few could do so. I learned how to perform DNA melting curves, and of course, there was the experiment to determine if we could actually "see" F-DNA. We did (11)! Julius remained a strong force throughout my career until his death. He would send me notes and journal articles inscribed with "You should look at this!"

I used my newfound molecular tools to study *E. coli*, *S. typhi*, and their hybrids. I hoped to understand how pathogenic enteric bacteria differed from their nonpathogenic brethren. I thought pathogens must have genes that defined their ability to be invasive; however, I could not establish this experimentally in *Salmonella* or in experiments I did with Sam Formal in *Shigella*. Thus, in 1962 when I presented my data on *Salmonella* and *Shigella* virulence to an audience at Cold Spring Harbor, their response was outright skepticism. Pathogens were seen as degenerate forms of bacteria that grew at the expense of the host and caused damage by doing so. I argued that pathogenicity was a kind of genetic adaptation for survival; pathogens were not all retrograde microbes or some kind of vicious throwback. I lacked the experimental data to make the point, and moreover, I didn't know what experiments to do to unequivocally demonstrate my view or to convince others that pathogenicity was not equivalent to disease.

I was counseled by my elders at Cold Spring Harbor to forget pathogens and to concentrate more on episomes, like F, and to continue the kind of work I had done with Marmur. By a fortunate accident (such an important ingredient in research), I noted that the newly described R factors, extrachromosomal elements mediating antibiotic resistance, could be seen as a faint band in a CsCl gradient of *Serratia* that carried these genetic elements. Therefore, I decided to focus my research on F and especially the R factors.

I established a close working relationship with two investigators, Naomi Datta at the Royal Postgraduate Medical School in London and Tsutomu (Tom) Watanabe of Keio University School of Medicine in Tokyo, who were working on the genetics of R factors. Our contact was by handwritten letters that detailed our respective progress, thoughts, suggestions, and ideas. It is pleasing to occasionally reread our correspondence and think about how earnest we were, how ignorant we were in some cases, and actually how insightful we were occasionally without realizing it. The problem then and now is most often we don't know which thoughts are ignorant and which thoughts are insightful until some time later.

In contemporary science, most PhD recipients spend three or more years as postdoctoral fellows in another laboratory. This is a time to put into practice the principles learned in pursuing the doctorate and permits a young investigator to become independent and to decide

what facet of science to focus on in the decades to follow. I did not have a formal postdoctoral education. Rather, I had been put into a relatively independent research position while I was still a doctoral student. Fortunately, I was invited to join a group at the National Institutes of Health (NIH) that became known as the Lambda Lunch Group. (It still exists today.) It began as an informal group, organized by Gordon Tompkins in the Laboratory of Molecular Biology (LMC), then part of the National Institute of Arthritis and Metabolic Diseases.

The world of molecular biology was making a transition from the bacterial genetics of the Pasteur school to the detailed molecular analysis of more-defined genetic elements with small chromosomes such as the bacteriophage lambda (λ) and, thank goodness, R factors. Thus, on Wednesdays, Lambda lunchniks, which included Gary Felsenfeld, Martin Gellert, Bob Martin, and Bruce Ames, met to listen to a talk or discuss a recent paper. A. L. (Larry) Taylor attended, as well as Gerry Fink and John Roth. All of them, like me, were just beginning in the field. The interactions with the attendees at the Lambda Lunch stood me in good stead for many years thereafter. Also, at NIH I met people like Wally Rowe, Maxine Singer, Malcolm Martin, Bill Hoyer, and Marshall Nirenberg, who answered my questions, explained things, or simply encouraged me with a gentle push and, on occasion, shove to explore new facets of science. Thus, although I didn't have a formal postdoctoral experience, I always thought myself lucky because, rather than having one postdoctoral mentor, I had many.

At one Lambda Lunch I heard about experiments performed by Ellis Bolton and Brian McCarthy at the Carnegie Institution of Washington's Department of Terrestrial Magnetism (DTM) using DNA immobilized in agar gels to quantitatively measure DNA-DNA and DNA-RNA hybrids. With their help I began to measure the relatedness between different bacteria, their viruses, and other extrachromosomal elements with reasonable quantization. I say this now, when the entire sequence of DNA for almost all pathogenic bacteria is known and stored in a public database and a simple computer command will compare two organisms, base by base, for their similarity. But in 1963, I was ecstatic. This simple experimental tool, together with the other methods I had learned, formed a tangible research plan to study pathogenic bacteria and their episomes and to determine how (if) they differed significantly from nonpathogens. Soon DNA agar columns became passé as we learned that certain kinds of filter paper sufficed, and the Southern blot revolutionized the way scientists looked at DNA homology. Now, of course, we have DNA and RNA microarrays to look at global relationships over entire genomes.

My WRAIR colleagues, John Wohlheiter and Ron Citarella, and I put our collective energy into examining the nucleic acid of F factor, R factors, and other extrachromosomal elements. In 1965 we characterized F-factor DNA (9), and a year later we published a broad study of the molecular nature of R factors (10). From 1965 to 1967, I collaborated with a number of different investigators at WRAIR, NIH, and Carnegie. Gary Felsenfeld and Shalom Hirschman at NIH introduced me to the fine mathematical details of analyzing the melting curves of DNA to gain insight into the evolution of bacteriophage genomes. This was complemented by work I did with Dean Cowie at Carnegie on the same subject. David Kohne, David Kingsbury, and Don J. Brenner were all postdoctoral fellows at Carnegie. Brenner and I worked together for the next seven years, measuring the molecular relationships between members of the *Enterobacteriaceae* using nucleic acid hybridization (2). Don eventually went to the Centers for Disease Control and Prevention (CDC) and was a major force in bacterial taxonomy and medical bacteriology.

GEORGETOWN UNIVERSITY (1967–1972)

Arthur Saz asked me to teach a few lectures to the medical students at Georgetown University.

I discovered teaching was as much fun as research. Saz persuaded me to move across town to join the Georgetown faculty in 1967. The National Science Foundation awarded me my first grant to study "bacterial extrachromosomal elements." I found people who were willing to work with me. Richard Silver, Pat Guerry, Lucy Tompkins, and Vickers Hirschfeld were my first graduate students. My first postdoctoral student was Don LeBlanc. We worked in a laboratory that was about as wide and half as long as a bowling alley lane into which we crammed the ultracentrifuges, liquid scintillation counters, and other equipment necessary in those days to do molecular biology. We began to look at the molecular events associated with the transfer of R-factor DNA into recipient cells using a method developed by David and Dorothy Freifelder. The university environment and the constant questioning by students validated my decision to follow a career of scientific research that had as its foundation teaching others.

I made more than my fair share of mistakes as a young professor. I was still fortunate to have a new set of mentors. Saz talked with me daily and managed to insert Talmudic-like wisdom and subtle and not so subtle messages into our conversations. Another person who influenced me was the dean of the medical school, John C. Rose, who seemed to have a perpetual little smile (not common for deans then and certainly not now). John helped me with my first steps in being a mentor.

The mentor's role is that of an advisor or counselor. It cannot be a friendship in the usual sense of the word nor can it be paternalistic. In so far as is possible, the advice should not be formulated into what would I (the mentor) do, but rather what would be in the best interest of the person I counsel. It requires absolute honesty and trust. It is a learned role and often the learning is through understanding the hopes, aspirations, and pain of the people who are your students. You must listen! Alas, I learned through mistakes made with my students. Errors are forgivable, especially if you recognize the error quickly and apologize. Most of all, try not to repeat the error again.

The most profound change for me as a professor was the realization that the research work would no longer be done with my own hands but by others. I had difficulty following the directions of others when it came to my own experiments. I did not feel that I could tell others what to do. My students could not be an extension of me. They had to follow their own ideas. I could be a dedicated participant and guide, but the decisions had to be theirs. This is not to say I didn't argue, cajole, whine, and even beg a student to do it my way. In the end, it was their decision. In the paragraphs that follow, it should be explicitly understood that it is really their story to tell and not mine. It has been a journey of shared hopes and ideas.

Saz and Rose helped me organize a symposium on R factors at Georgetown. I invited and finally met Tom Watanabe and Naomi Datta, as well as Pete Guinée from the Rijksinstituut De Volksgezondheid in Utrecht, The Netherlands. I also invited Bob Rownd and several U.S. infectious diseases specialists including David Smith and Vernon Knight to present their work and their ideas on this new research area. This was the first international meeting on the subject of transferable antibiotic resistance, and as a consequence I received a number of invitations from around the world to speak about my work.

The invitations were a major problem for me because for many years I had a dread of flying and travel in general; however, I managed to overcome these phobias and attended the Ciba symposium on extrachromosomal elements in London in the fall of 1968. This meeting was memorable because I had the chance to meet such legendary figures as William Hayes, Eli Wollman, and Werner Arber. I met my own contemporaries like Richard Novick, who was just beginning his pioneering studies on staphylococci. I became reacquainted with Allan Campbell and spent several hours receiving helpful guidance from Salve Luria. This meeting was notable because it officially blessed the term plasmid to replace the term episome (25).

I suppose these memories of my first important scientific meeting are of little consequence to anyone other than me. In reading my story, it may be useful for you to understand that because of my panic attacks, I had been living my life both scientifically and personally in a kind of cocoon, always half afraid and ready at a moment's notice to run. A newfound freedom crystallized at this meeting. As much as this meeting was an important personal milestone, it also had an important scientific impact as well.

Naomi Datta asked me to join her and H. Williams (Willie) Smith at a pub for a drink. Willie, a veterinarian, was interested in the work I presented on the molecular nature of R plasmids and asked if it could be extended to other plasmids. I was unaware of his work and asked Willie what plasmids he had in mind. In the minutes that followed, I was treated to an unaffected explanation of one of the most exciting things I had ever heard in medical microbiology. In his soft Welsh accent, he told me that *E. coli* were the cause of an infectious diarrhea in pigs and other farm animals. He described marvelous experiments that established that the epidemic *E. coli* strains carried a transferable plasmid that encoded one or more enterotoxins (23). He further astounded me by saying a second plasmid was necessary for the bacteria to adhere to the pig's intestinal cells. He offered to send me any or all of his strains and said simply, "Just promise me you'll let me know what you find." I was at the time drinking my first pint of English bitter, and this gracious offer caused me to try to swallow the stuff and say yes simultaneously with somewhat embarrassing consequences for my shirt and innocent bystanders. Willie was a great scientist and we enjoyed a long relationship exchanging a number of letters about our research and slanderous gossip.

I returned to my laboratory at Georgetown resolved to extend our studies to these novel elements, which, wondrously, wedded plasmids and pathogenicity. Shortly thereafter I met Sherwood (Sherry) Gorbach, who had human *E. coli* isolates that produced enterotoxins like those described by Smith. The human strains came from travelers, and Gorbach regaled me with stories of the history of Montezuma's Revenge, guppy tummy, and traveler's quickstep. Yet, this was no laughing matter where the same disease was a leading cause of death in infants and toddlers of developing countries. We showed that one *E. coli* strain harbored three plasmids, one of which encoded an enterotoxin very much like those encoded in the pig strains. However, working with enterotoxin plasmids and the plasmids encoding adherence factors was not as straightforward as working with R factors. There was no selectable marker.

Thanks to seminal work done by E. S. Anderson at the Enteric Reference Laboratory of the Central Public Health Laboratory in London, and Roy Clowes at the University of Texas, Dallas, it was recognized that not all R plasmids were transferable. Rather, quite small molecular species encoding resistance could be mobilized from bacterial cell to bacterial cell by a transmissible plasmid such as F, or in our case Ent, which did not itself confer a resistance trait. This concept of plasmid mobilization was an important concept for understanding plasmid epidemiology. Small plasmids like pSC101 and RSF1010 carrying only resistance genes and a simple replication apparatus became the foundation of the cloning vectors of the future. Naomi Datta and her associate, Bob Hedges, began to classify R plasmids on the basis of whether or not two plasmids could coexist in the same cell (4). If the plasmids could not be coresidents (compatible) in a bacterium, they considered the plasmids to be incompatible. Bob Hedges asked us to take representative examples of each incompatibility class to tell him about their molecular properties. Pat Guerry and I established in 1970 that a human enterotoxin plasmid and Willie Smith's pig Ent plasmid were related to the classic F factor. We included in this study R plasmids from two other incompatibility groups called I and N. These plasmids exhibited virtually no homology to each other, even though they mediated resistance to the same antibiotics. Clearly the bulk of the plasmid genes, those probably used for replication and transfer, were not related. But

Julian Davies and his coworkers showed that different kinds of aminoglycoside antibiotics were neutralized by specific R-factor-encoded enzymes and the enzymatic mechanism of resistance for streptomycin for the N plasmid was the same as it was for the I incompatibility group R plasmid we studied.

SEATTLE (1972–1981)

While I was in the midst of juggling R factors and Ent plasmids and wondering about their evolution, Brian McCarthy called to say he was at the University of Washington, Seattle, and asked if I was interested in applying for a job there. The Department of Microbiology in Seattle was, and is, among the very best in the United States. The faculty was legendary and included the Chair, John Sherris, as well as Helen Whiteley, Neal Groman, Erling Ordell, Howard Douglas, Charles Evans, Russell Weiser, and Eugene Nestor. The negotiations were brief, and on the evening of the Watergate break-in, June 17, 1972, my wife, two daughters, and I boarded an airplane at Dulles and, with two very angry cats tucked away in the baggage hold, moved to Seattle.

The move to Seattle provided me with more resources plus another bonus. The University of Washington environment permitted me to work again with the hospital microbiology laboratory. Every day the clinical microbiology laboratory held an hour-long session called plate rounds. Fritz Schoenknecht and the eminent physician William (Bill) Kirby presided over these meetings. They had a stunning breadth of knowledge about all microorganisms that might be cultured from the human body. I always thought that plate rounds provided me with a unique view of the natural history of microorganisms that inhabit humans. I don't suppose it is fair to say it is akin to what Darwin did while he wandered the Galapagos looking at finches. But, in a way, I learned that even things that seem to be the same, like *E. coli* from a stool sample or urine culture, are often different if you look closely enough, just as the beaks of the Galapagos finches were different in length and revealed something of their biology.

Thanks to King Holmes, Marvin Turck, and the late Bob Petersdorf, I was given a joint appointment in the Department of Medicine and became a participant in the Infectious Diseases (ID) Training Program. The young ID faculty included Larry Cory and Walt Stamm, who have remained close friends. This relationship led to a steady stream of infectious diseases physicians in training in my laboratory over the next decade. Among the first of these were Dennis Schaberg, Peter Piot, Lucy Tompkins, and Michael Lovett. Infectious diseases physicians also arrived as sabbatical visitors and included Mitchell Cohen from the CDC and Rainer Laufs from the Hygiene-Institut der Universität Göttingen, Germany (now at the University of Hamburg). They worked beautifully and productively with the graduate students and postdoctoral fellows who joined me in Seattle. This blend forever after became an important feature of my laboratory.

My first order of research in Seattle was to focus on the enterotoxin plasmids. We became more involved with learning the assays to detect enterotoxins. Fortunately, Carlton Gyles, a veterinarian who had worked with Willie Smith, arrived in Seattle as a sabbatical visitor, and he played a major role in our effort. In the beginning, my Seattle laboratory included Jorge Crosa; graduate students Magdalene (Maggie) So, Fred Heffron, and Leonard Mayer; and Pat Guerry, who came with me from Georgetown to work as a research associate. In November of 1972 I traveled to Honolulu to participate in the U.S.-Japan Cooperative Conference on Bacterial Plasmids. I have written about an informal evening snack at a delicatessen where Herb Boyer, Stan Cohen, Charlie Brinton, and I discussed the possibility of splicing together DNA from different sources (8). Several months after the meeting, Herb Boyer called to tell me that the initial splicing experiments linking together pSC101 and RSF1010 worked. We agreed that Maggie So would travel to Herb's lab to try to "clone" the *E. coli* Ent plasmid enterotoxin genes.

It might seem that the discovery of gene splicing would have had an immediate and profound impact on our work. Eventually it did, of course, but it was still necessary to characterize the DNA! We couldn't yet characterize large chromosomes, so we focused on our beloved plasmids. Characterization was elementary but laborious. We first had to separate the few percent of plasmid DNA from the total bacterial DNA by density centrifugation. We measured the molecular mass of the plasmid DNA of interest by sucrose gradient sedimentation. My new colleagues and friends at Seattle, Jimmie Lara and his wife, Stephanie, taught me how to use the electron microscope to photograph circular DNA and to measure its contour length to estimate the molecular mass of plasmid DNA. It was this tedious approach that permitted Lynn Elwell and Hans de Graaf to characterize the R plasmids in *Haemophilus influenzae* and Leonard Mayer to catch a glimpse of the indigenous plasmid of the gonococcus.

Guerry, Crosa, and I were in danger of drowning in a sea of different sized plasmids; however, we were also refining our skills at performing DNA-DNA reassociation experiments, and the smaller plasmids we found that encoded one or two antibiotic-resistant traits began to reveal something about the distinction between the replication machinery of a plasmid and the mysterious origin of the carried genes encoding antibiotic resistance and enterotoxins. Using hybridization experiments, an undergraduate, Richard Sublett, showed that the ampicillin-resistant genes found on diverse plasmids were often identical. I sent these results to Datta and Hedges by airmail, and a packet from them crossed in the mail containing the draft of a manuscript by Alan Jacob and Hedges describing DNA transposition of ampicillin resistance (14).

Fred Heffron became excited by these findings, and we proposed to look at their plasmid carrying the translocated ampicillin by using the DNA-DNA heteroduplex method in the electron microscope as had been done by Phil Sharp in Norman Davidson's lab. One Saturday morning in 1975, it fell to me to sit down at the microscope to search for the telltale molecule—two identical plasmids except for a single region that would appear as a kinky insertion loop (15). My feeling when that first molecule came into view remains one of the most exciting moments in my scientific life. Fred and I danced around the lab to quizzical stares. Fred, Ron Gill, Craig Rubens, and Pat Guerry went on to publish a wonderful set of papers on transposon biology. At virtually the same time, Dennis Kopecko and Stan Cohen, Nancy Kleckner and David Botstein, as well as Doug Berg and Julian Davies, described similar findings using other antibiotic-resistant genes.

The field of recombinant DNA became a hotbed of political activity. The publication of an open letter to the scientific community stating that there should be a moratorium on several forms of experiments brought about a meeting at Asilomar, California, in 1975. A group of us who originally had been asked to help bring some formality to naming plasmids (how many R1 plasmids could the literature tolerate?) (21) were pressed into action to provide guidelines for recombinant DNA experiments involving bacteria, their plasmids, and their phages. Much has been written of the Asilomar meeting (18), and I can add little to that extensive history.

Roy Curtiss, Don Helinski, and I were appointed to the first NIH Recombinant DNA Committee and charged with helping to prepare formal experimental guidelines. This too has been the subject of many historical books and papers (13). I have nothing to add to this except to pay homage to Bill Gartland and Dewitt (Hans) Stetten, Jr., who were the NIH's stewards for the public good. I resigned from the Committee once the first guidelines were adopted to turn my attention to service on a Food and Drug Administration (FDA) committee concerned with antibiotics and animal feed. I was a strong proponent for the removal of antimicrobials in animal feed. Donald Kennedy, who led the FDA in the late 1970s, proposed to remove or strictly control many antimicrobial agents in animal feed and fell into a buzz saw of controversy.

These nonlaboratory facets of my professional life were time consuming. I never minded serving on study sections or editorial boards but this was different. In retrospect I learned a good deal about the interconnection of our political system with science. I will never forget the times I testified before Congress and I even testified before the city council of Cambridge, MA. I also learned that I didn't want to do this if I could avoid it. Yet, it is also a scientist's responsibility to serve the public's interest.

During this same period, I also witnessed the birth of biotechnology. Stan Cohen and Herb Boyer applied for patents on the gene-splicing technology through their respective universities. Herb took the first steps to launch what became Genentech, while Stan Cohen was involved with another company called Cetus-Palo Alto. Over the years, I served as a scientific advisor for a number of biotechnology companies, and I sometimes laugh at how naive we were about turning a scientific idea into an actual product. In the years that followed, I have been privileged to watch the evolution of an extraordinary industry worldwide and to see the application of those primitive ideas become products that have a major impact on people's lives and well-being. I have always thought that biotechnology was the direct result of the research funding philosophy of 1950–1980, which was to encourage individual creativity and to invest in the best people no matter what their precise area of scientific expertise might be.

The recombinant DNA controversy underlined the need for safe cloning vectors and host bacteria. Crosa and I worked closely with the Boyer lab and especially Francisco "Paco" Bolívar to produce vectors that became known as pBR313 and pBR322 (1). An unexpected fallout from this work was the serendipitous discovery that agarose gels could be employed to analyze the plasmids of bacteria (19). We could abandon density gradient centrifugation, sedimentation in sucrose gradients, and examination of circular DNA in the electron microscope. We could look at simple lysates electrophoresed through agarose to instantly tell the number of different plasmid classes in a microbe and their molecular mass from the distance the molecules migrated into the gel. This simple procedure led to the rapid discovery of the nature of R plasmids in the gonococcus by Marilyn Roberts and Lynn Elwell, to the first plasmid epidemiology studies by Dennis Schaberg, Lucy Tompkins, and Jim Plorde, and to the use of restriction polymorphisms by Jim Kaper in his studies of cholera vibrios from Chesapeake Bay and Louisiana.

We continued to make progress on characterizing the enterotoxin plasmids of *E. coli*. Maggie So cloned the *E. coli* ST enterotoxin. Walter Dallas cloned the heat-stable enterotoxin, LT, and introduced DNA sequencing into the laboratory. Steve Moseley did seminal work on the use of DNA hybridization to detect pathogens directly in clinical material using enterotoxin genes as probes. However, I had determined, almost from the moment that I understood the power of recombinant DNA methods, to begin to search once more for the determinants of bacterial pathogens that distinguished them from the more numerous harmless bacteria that inhabit humans.

I took a sabbatical leave in 1978 at the University of Bristol in Mark Richmond's laboratory. I stopped work on R factors in the laboratory. I had been a member of the R-plasmid research community for 18 years, and we continued to interact even as our paths took divergent turns in the years that followed. I remain indebted to my friend, Julian Davies, for his steady good counsel, for teaching me about the ecology and biochemical basis of drug resistance and that "life is too short to drink bad wine."

When I returned from England there was an assembly of new graduate and postdoctoral students waiting who were encouraged to explore aspects of bacterial pathogenicity. Dan Portnoy, with help from Moseley, began to examine the plasmids of *Yersinia*. Michael Koomey cloned the IgA protease gene from the gonococcus. I accidentally met the wonderful Margaret Pittman at a meeting and her first words to me were, "If you're so smart, why aren't you working on pertussis?" I made

the same challenge to Alison Weiss and she surpassed Margaret's dreams in the years to come. Richard and Sheila Hull translated the phenotypes observed by Barbara Minshew and Catherina Svanborg in uropathogenic *E. coli* into cloned virulence genes.

STANFORD UNIVERSITY (1981–PRESENT)

In 1981 I moved to Stanford University as the Chair of the renovated Department of Microbiology and Immunology and was charged with recruiting young faculty and revamping the teaching program. I was able to recruit John Boothroyd, Ed Mocarski, and Mark Davis as the first faculty members of our young department. I taught most of the medical bacteriology lectures during those first years at Stanford, with an emphasis on syndromes rather than using taxonomic groups of organisms. Subsequently, Gary Schoolnik, Ann Arvin, and Harry Greenberg, physicians in clinical departments who also had active basic research interests, accepted a joint appointment in the department as an adjunct to our teaching effort. They were, and continue to be, active in teaching and as graduate student mentors, as well as collaborators in research efforts of common interest.

The move to Stanford had a profound impact on the structure of my laboratory. At both Georgetown and the University of Washington most of the members of my laboratory were graduate students. The graduate program at Stanford was quite small by comparison, and there was a greater emphasis on postdoctoral students. The original cadre of graduate students working on pathogenic determinants traveled with me to Stanford. They were joined by Paul Orndorff, Rod Welch, and David Low as postdoctoral fellows. They selected their research projects from the panel of the cloned genes that the Hulls had isolated from uropathogenic *E. coli*. Orndorff took the clone with the type 1 pilus genes, while Welch and Low selected a cloned sequence that mediated the *E. coli* α-hemolysin (24).

I was especially lucky that first year to have Staffan Normark as a sabbatical visitor. He, like Gordon Dougan and Mark Achtman, who were visitors in Seattle, had decided to change his focus from antibiotic resistance to the study of pathogenicity. Staffan's presence was a constant stimulus not only for his intellect and humor but because he provided a steady stream of visitors like Hans Wolf-Watz from his department at Umeå University in Sweden. It is noteworthy, I think, that when Staffan returned to Umeå, he was so excited about bacterial pathogenicity that he organized an EMBO course on the genetics and molecular biology of pathogens in the summer of 1983. The course was the first of its kind in Europe and attracted a number of participants, many of whom were established investigators in other aspects of microbiology.

The course faculty reflected the youth of the field and included Staffan, Dan Portnoy, Michael Koomey, Alison Weiss, Maggie So, and John Mekalanos. John, when he was a student, had worked for a few weeks in our lab in Seattle on enterotoxins and had now embarked on his career to study *Vibrio cholerae*, its toxin, and its regulation. I joined the course faculty for a five-day period at the end of the course and got to know a number of the participants, especially Rino Rappuoli and Jörg Hacker. I have been told that Staffan's dream course was the launching pad into the bacterial pathogenesis arena for many European scientists.

Alison Weiss departed for her postdoctoral studies, and *Bordetella pertussis* attracted the next generation of students. Scott Stibitz began work on virulence regulation. Bill Black worked on the contribution of pertussis toxin to virulence. David Relman traveled to Siena, Italy, to work with Rino Rappuoli's group to characterize the filamentous hemagglutinin. Subsequently, Jeff Miller and Craig Roy did seminal work on the control of *Bordetella* virulence regulation by a gene they named *bvg*.

I have never been a politic man. At university meetings, it always seemed I had half the people smiling and the other half scowling. I resigned as the Chair in 1985 to return to a life in the laboratory where, all agreed, I was better suited.

The era of research on uropathogenic *E. coli* ended by 1988 in the laboratory with the studies by Agnès Labigne on afimbrial bacterial adhesins and Carleen Collins' work on the role of bacterial urease in bladder infection. Also while Mike Lovett, Lindy Palmer, and Patrick Bavoil had worked diligently on aspects of *Chlamydia trachomatis* pathogenesis, I banned further work on it and *Neisseria gonorrhoeae* despite Jay Shaw's work on entry into epithelial cells by invoking the "it will break your heart" rule. If we were to study a microbe in the laboratory, it had to be reasonably easy to grow, it had to have a genetic transfer system available (or likely available), and there had to be a suitable animal model of infection. The "it will break your heart" rule was undoubtedly shortsighted, not necessarily strictly followed, but, all in all, it worked to save me at least a good deal of frustration.

The era of bacterial invasion began in the laboratory as the work on pertussis and uropathogenicity waned. Pamela Small, a graduate student, established invasion assays for *Yersinia* and *Salmonella* in epithelial cells and introduced us to the use of gentamicin to kill extracellular bacteria, while sparing their intracellular brethren. Ralph Isberg came to the laboratory with the express idea of cloning genes important for bacterial invasion. He chose *Yersinia pseudotuberculosis*. With the simplicity that so often accompanies a fundamental scientific discovery, Isberg cloned a single genetic determinant he called invasin that transformed ordinary *E. coli* K-12 into a microbe that could easily breach the epithelial barrier, even though this extraordinary trait is not itself sufficient to make the organism pathogenic (17). Virginia Miller found other such genes she called *ail* that encoded not only invasion but also serum resistance. These discoveries by Isberg and Miller pointed out our lack of expertise in studying even the most rudimentary aspects of host cells.

The NIH asked me to help review the Rocky Mountain Laboratory (RML) in Hamilton, Montana. There I encountered Claude Garon from my Georgetown days. At the time, I had just experienced a divorce, and at age 50, I was thinking about what had been missing in my life outside of science. Fly fishing was one such passion I had ignored, and I rediscovered its joys in the Bitterroot River that flowed behind RML. Claude, an expert electron microscopist, encouraged me to come back to RML the following summer to learn how to use microscopy to study infected animal cells. So in the summer of 1986, I took my first micrographs of *Yersinia* invading animal cells at RML. Virginia Miller looked at my first attempts and remarked, "Oh, Stanley, these will never do!" I did get better, and every year since then I have returned to the Bitterroot Valley to explore science and, on occasion, the joys of fly fishing and even took a few EM photographs in focus.

Brett Finlay initiated work on the entry of *S. typhimurium* into polarized epithelial cells. I had given up on *Salmonella* in 1967. Twenty years later he brought it back into my scientific life. Brett used this approach to isolate bacterial mutants defective in entry, and in doing so, with his characteristic enthusiasm, changed the flavor of the laboratory forever by introducing us to many of the fundamental methods used by cell biologists (12). I began to seek out cell biologists like Ari Helenius, Ira Melman, Suzanne Pfeffer, and Kai Simons, who were uniformly enthusiastic and helpful. The work on *Salmonella* quickly expanded. Cathy Lee pursued the regulation of *Salmonella* invasion. Bradley Jones extended Lee's work and also established, with Nafisa Ghori, a model to look at the entry of *Salmonella* into the Peyer's patch through M cells. Carol ("Connie") Francis, a graduate student, and I spent some time together as "students" in adjacent Stanford laboratories run by Stephen Smith and James Nelson where we learned in vivo imaging, confocal microscopy, and the rudiments of epithelial cell biology. Carol, with help from Michael Starnbach, was thrust into the world of cell ruffling and the intricacies of the host cell cytoskeleton. Although epithelial cells were fine experimental tools, it was clear from the histopathology we began to perform on infected animals that our current favorite organisms, *Yersinia* and *Salmonella*, were much

more likely to be found in the host macrophages than in epithelial cells. Fortunately, RML purchased a confocal microscope, and in 1990 I began to apply my newly learned skills to help Michelle Rathman follow the trafficking of internalized *Salmonella* in macrophages.

Of course we were not alone in our pursuit of examining the precise interactions of host cells and microorganisms. Frequently I ran into the always enthusiastic Jorge Galán, my good friend Philippe Sansonetti (we shared Sam Formal as our mentor and are recent fellow aviators), and the irrepressible Pascale Cossart at meetings, where we compared notes on our burgeoning interests in cell biology.

Pascale, together with Rino Rappuoli, Pierre Bouquet, and Staffan Normark, organized a meeting that was jointly sponsored by the American Society for Cell Biology and EMBO on epithelial cell cross-talk between microbes and host cells in August 1991 in Arolla, Switzerland. This meeting was an attempt to get the two cultures of cell biology and those interested in microbial pathogenesis to talk with one another. I think it worked well and had a seminal effect on the field. It helped launch the journal *Cellular Microbiology* and a plethora of subsequent meetings on host cell–pathogen interactions (3).

From 1988 to 1995 there were new experimental systems coming into play in the laboratory seemingly from all directions. Or, perhaps it was my lack of direction. It was during this time I also had a glimpse into the future that in many ways paralleled the experience I had when I understood what the impact of gene cloning would have on the future of science. Bill Haseltine invited me to visit his company, Human Genome Science, in 1992. Bill suggested we stop by the nonprofit arm of the company, the Institute for Genomic Research, directed by Craig Venter. We arrived after 6:00 p.m. on a warm summer evening. There was no one there, but I gasped when I saw the rows of DNA sequencing machines, all connected by an umbilicus of cables that disappeared into the ceiling. We followed the cables to a dimly lit room that contained computers and a row of printers spewing ATGC in a rapid staccato. My actual response cannot be printed here, but as Bill explained to me the scope of the project and what Venter envisioned was possible, I was stunned. When I called home that evening, I told my wife, Lucy, I had just seen the future and science would be practiced differently in the very near future.

However, the impact was not immediate in my laboratory. There was a second wave of research on the *Yersinia* pursued by Kathleen McDonough, who began to haunt the ground squirrel dens of Stanford to harvest fleas to infect with the plague bacillus. Dorothy Pierson began to explore the function of the *ail* gene. Jim Bliska, working closely with Jim Dixon's laboratory, soon began to characterize the effect of YopH, tyrosine phosphatase, on macrophages, while Joan Mecsas and Bärbel Raupach compared the different strategies used by *Yersinia* to exploit the host cell cytoskeleton. At the same time Denise Monack began to work on the cellular basis of pathogen-induced cytotoxicity induced by *Yersinia* and *Salmonella*.

New organisms somehow kept appearing! Joe St. Geme finished his training in pediatrics and began to explore the interaction of *H. influenzae* with epithelial cells. David Schauer, a veterinarian, earning his PhD, taught me about the *Citrobacter* species in mice that caused colonic hyperplasia and showed it was a variation on a theme of enteropathogenic *E. coli*. David Relman and I attended an infectious diseases conference on a day when we had discussed a paper by Norm Pace on exploring bacterial communities using 16S RNA probes. We heard about a bacterial disease of patients infected with HIV called bacillary angiomatosis, and he, together with Lucy Tompkins, used the 16S approach to show that the agent was a *Bartonella*.

Lalita Ramakrishnan, another ID fellow, was determined to work on *Mycobacterium tuberculosis*. I told her I was too old to wait a month for a colony to grow. She convinced me to let her focus on the faster-growing *Mycobacterium marinum* instead. I kept getting talked into things! Evi Strauss worked on *Edwardsiella*

tarda; Brendan Cormack wanted to work on *Candida* pathogenesis. I told him I knew nothing about fungi; he told me he knew nothing about bacterial pathogenesis. We'd teach each other. He did far better than I. Creg Darby looked at *Caenorhabditis elegans* interacting with *Y. pestis* and found a mechanism that probably accounts for how the plague bacillus blocks the foregut of fleas.

The serendipity of scientific discovery was always our partner. Raphael Valdivia planned to do a thesis project on intracellular trafficking in *Mycobacteria* but became enchanted with the green fluorescent protein (GFP) of a jellyfish. He and Cormack, almost as a lark, planned an experiment that provided a marvelous probe for many investigators in different scientific disciplines. Valdivia instead used GFP to isolate genes expressed only inside of macrophages.

The inevitable departure of people to establish their own laboratories led to other changes. There was a decided increase in individuals who had initially worked in eukaryotic systems and who now wished to study bacterial pathogenesis. Most of these people felt that the microbe could be used as a tool to study the biology of the host. I felt that one had the best of both worlds. You learned as much about the pathogen as you did about the host.

In the last decade my lab was active, our work focused primarily on two pathogens. One was *Salmonella*, my first love. The *Salmonella* work was fueled by Valdivia's discovery of specific genes expressed intracellularly. This work appeared shortly after David Holden's description of signature-tagged mutagenesis and his discovery of the Spi2 pathogenicity island (16). Also, Denise Monack established a persistent infection model in *Salmonella*, which more closely resembled the natural disease seen in typhoid with its hallmark asymptomatic bacterial shedding. Anthea Lee, Corrie Detweiler, and Igor Brodsky looked specifically at some of the Spi2 genes and how they were regulated and functioned in the phagosome.

The second focus of the laboratory became *Helicobacter pylori*. The reasons for this are rather more personal. Lucy Tompkins and I were married in 1983. She had gone from her PhD work in my laboratory in 1967 to Dartmouth Medical School, and subsequently she did her infectious diseases residency at the University of Washington. After our marriage, she moved to the Division of Infectious Diseases at Stanford and established her own laboratory. A few years ago, Lucy was asked to become the Chief of the Division of Infectious Diseases and Geographic Medicine, as well as an associate dean. This inevitably took away the time she could spend in the laboratory. She convinced me to become involved with her and her research associate, Ellyn Segal, to look at *H. pylori* pathogenicity. Ellyn had discovered that the CagA protein caused human cells to elongate and this was accompanied by a phosphorylation event. By going to meetings with Lucy, I knew many of the major investigators in this arena, including Barry Marshall, Marty Blaser, Jeffrey Gordon, and Antonello Covacci. The first time I heard Barry Marshall talk about the isolation of *H. pylori* and his view of its role in gastritis and gastric cancer, I stated unequivocally that I thought it was nonsense based on two misguided physicians drinking a bacterial culture and becoming ill. I confessed my error in public when Barry asked me to deliver one of the major talks at a symposium celebrating the twentieth anniversary of his discovery. *H. pylori* has charisma and many of the students with prior experience in eukaryotic biology were drawn to study it because of its association with gastric cancer.

The genomic revolution touched the laboratory owing to the urging of my Stanford colleague, David Botstein, to forge ahead and use Pat Brown's DNA array technology to look at global sequence homology and global gene expression in host-pathogen interactions. We turned first to *H. pylori* because it possessed a relatively small genome. Nina Salama and Tim McDaniel bravely set out to construct a DNA array of the *H. pylori* genome. Nina studied the phylogeny of *H. pylori* and used the array to define the organism's essential genes. Lucy Thompson, an Australian graduate

student, used the array to explore *H. pylori* physiology. On the other side of the coin, Karen Guillemin was involved with the construction of a mouse and a human DNA array. Karen examined the effect of *H. pylori* infection on eukaryotic cells using a human DNA array. Her findings unexpectedly revealed that a large number of genes associated with the tight junctions were upregulated when *H. pylori cagA* gene product was injected into cells. Manuel Amieva, Roger Vogelmann, and James Nelson went on to show how the insertion of CagA into host cells caused cytoskeletal rearrangements and triggered a number of cell signaling pathways. These findings were extended by Scotty Merrell and Sahar El-Etr using human polarized cells. Finally, Anne Mueller, working with our Australian collaborators Adrian Lee and Jani O'Rourke, did an in-depth analysis of the natural history of mucosa-associated lymphoid tissue (MALT) formation and its transition to lymphoma in mice over an 18-month period. Anne and Scotty went on to examine the dynamics of the infection process and introduced the laboratory to the use of laser capture microscopy coupled with DNA microarray analysis.

Despite my protestations about remaining focused these past years, it is true that I could always be convinced by someone to bring another microbe into the fold. Erin Gaynor initiated a study of *Campylobacter jejuni* with a graduate student, Joanna MacKichan. They somehow managed alone to construct a genome array and looked at virulence regulation in the somewhat ignored important enteric pathogen. Wanda Songy tried to discover why certain *Streptococcus bovis* are found in sepsis in colon cancer patients. Last, but not least, Elizabeth Joyce, even today, is trying to understand how the pneumococcus colonizes the nasopharynx.

Kaman Chan and Charlie Kim, my last graduate students, constructed a complete DNA array of *Salmonella* genes. Charlie published several bioinformatics tools while working on this project, as well as investigating nitrite-inducible *Salmonella* genes. Kaman looked at the molecular phylogeny of *Salmonella* and then developed a global method to look at *Salmonella* genes essential for survival in a macrophage during infection.

So, during these past few years, those working in the lab have moved more and more into the host arena and led me to appreciate more fully the nuances and intricacies of the host and pathogen interplay. It seems clear to me that the term bacterial pathogenesis places far too much emphasis on the microbe and downplays the impact of the host on the evolution of the pathogen. Indeed, it is now clearer than ever that the host-pathogen interaction has been one of the driving forces of eukaryotic evolution including ours; our total cellular burden is, after all, 90% microbial.

FINAL THANK YOU

I was a student during the first era of bacterial genetics. I witnessed the emergence of molecular biology. I witnessed and was a beneficiary of the discovery of messenger RNA, the genetic code, recombinant DNA technology, DNA sequencing, PCR, and most recently, genomic sequencing and now array technology and systems biology. I look back with fondness on the days, many years ago now, when we sought to discover the nature of plasmid resistance genes and the role of plasmids like Ent in the evolution of bacteria. I thought then it was the most important problem in science, which is how it should be when one is young. In the years that followed there grew an ever greater passion to understand the biology of pathogens, and it is fitting that pathogenicity is intricately enmeshed with mobile genetic elements not unlike R factors and transposons. Each day still brings up questions more important and more challenging than those from the day before, which is how it should be even as I grow older. My boyhood dream of a life of scientific adventure was surpassed by reality and human creativity, a human characteristic that has not been lost despite the poor record of human history.

I was sad to close my laboratory in 2005. Fortunately Manuel Amieva, Denise Monack,

and David Relman are now Stanford faculty members and still study fascinating organisms. They and their students keep me abreast of their latest findings and the newest developments in the host-pathogen research field. I was fortunate to have worked with so many talented and genuinely nice people in my career. They constitute a reservoir of wonderful memories, some of which I've tried to share with you. I still remain in contact with most of them. One never truly loses a former student. There is, at the least, one more letter to write no matter how old they or I become.

Finally, I realized a number of years ago that in life you never get to have as many dogs as you want or to find out how it all turns out. As you get older and are asked to be a keynote speaker or get an award of some kind, you also realize you are being given credit for things you never did and for attributes you never possessed.

ACKNOWLEDGMENTS

I should note that a number of my former students wrote articles about the early years in my laboratory in a volume celebrating my sixtieth birthday. I deliberately did not cite these because this book is not readily available to many people (7). However, it provides another perspective of some of the events I describe here.

Anyone who knows me at all has met my assistant, Sara Fisher. We have worked together for almost 20 years, and she has been the surrogate mother to most of the people who worked with me these past years at Stanford. She has been a mainstay of my professional life as well as my friend.

Lucy Tompkins, my wife, has been my best collaborator and introduced me to what love and joy can mean in life.

I thank Carleen Collins for reading this article and providing me with corrections and cautions that I treasure but did not always follow.

As noted above, after the manuscript was completed, I felt there was only one of my former students, Carleen Collins, who would tell me the unvarnished truth about what I had written. Tragically, just a few weeks later, Carleen was diagnosed with a very aggressive malignant disease and she passed away on February 12, 2008. I treasured her as a person, as a scientist, and as an advisor. I shall miss her as will all of her friends and colleagues.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Bolivar F, Rodriguez RL, Greene PJ, Betlach MC, Heyneker HL, et al. 1977. Construction and characterization of new cloning vehicles. II. Multipurpose cloning system. *Gene* 2:95–113
2. Brenner DJ, Fanning GR, Johnson KE, Citarella RV, Falkow S. 1969. Polynucleotide sequence relationships among members of the Enterobacteriaceae. *J. Bacteriol.* 98:637–50
3. Cossart P, Boquet P, Normark S, Rappuoli R. 1996. Cellular microbiology emerging. *Science* 271:315–16
4. Datta N, Hedges RW. 1971. Compatibility groups among fi-R factors. *Nature* 234:222–23
5. Falkow S. 1957. L-Forms of proteus induced by filtrates of antagonistic strains. *Bacteriology* 73:443–44
6. Falkow S. 1958. Activity of lysine decarboxylase as an aid in the identification of Salmonellae and Shigellae. *Am. J. Clin. Pathol.* 29:598–600
7. Falkow S. 1994. A look through the retrospectoscope. In *Molecular Genetics of Bacterial Pathogenesis*, ed. VL Miller, JB Kaper, DA Portnoy, RR Isberg, pp. xxiii–xxxix. Washington, DC: ASM Press
8. Falkow S. 2001. I'll have the chopped liver please, or how I learned to love the clone. *ASM News* 67:555–59

9. Falkow S, Citarella RV. 1965. Molecular homology of F-merogenote DNA. *J. Mol. Biol.* 12:138–151
10. Falkow S, Citarella RV, Wohlhieter JA, Watanabe T. 1966. The molecular nature of R-factors. *J. Mol. Biol.* 17:110–16
11. Falkow S, Marmur J, Carey WF, Spilman W, Baron LS. 1961. Episomic transfer between *Salmonella typhosa* and *Serratia marcescens*. *Genetics* 46:703–6
12. Finlay BB, Gumbiner B, Falkow S. 1988. Penetration of *Salmonella* through a polarized Madin-Darby canine kidney epithelial-cell monolayer. *J. Cell Biol.* 107:221–30
13. Fredrickson DS. 2001. *The Recombinant DNA Controversy: A Memoir. Science, Politics, and the Public Interest 1974–1981*. Washington, DC: ASM Press. 388 pp.
14. Hedges RW, Jacob A. 1974. Transposition of ampicillin resistance from RP4 to other replicons. *Mol. Gen. Genet.* 132:31–40
15. Heffron F, Sublett R, Hedges RW, Jacob A, Falkow S. 1975. Origin of the TEM beta-lactamase gene found on plasmids. *J. Bacteriol.* 122:250–56
16. Hensel M, Shea JE, Gleeson C, Jones MD, Dalton E, et al. 1995. Simultaneous identification of bacterial virulence genes by negative selection. *Science* 269:400–3
17. Isberg RR, Falkow S. 1985. A single genetic locus encoded by *Yersinia pseudotuberculosis* permits invasion of cultured animal cells by *Escherichia coli* K12. *Nature* 317:262–64
18. Lear J. 1978. *Recombinant DNA: The Untold Story*. New York: Crown. 280 pp.
19. Meyers JA, Sanchez D, Elwell LP, Falkow S. 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. *J. Bacteriol.* 127:1529–37
20. McElroy WD, Glass B. 1957. *The Chemical Basis of Heredity*. Baltimore: Johns Hopkins Press. 848 pp.
21. Novick R, Clowes RC, Cohen SN, Curtiss R III, Datta N, et al. 1976. Uniform nomenclature for bacterial plasmids: a proposal. *Bacteriol. Rev.* 40:168–89
22. Schaeffer AB, Fulton D. 1933. A simplified method of staining endospores. *Science* 77:194
23. Smith HW, Halls S. 1967. Studies on *Escherichia coli* enterotoxin. *J. Pathol. Bacteriol.* 93:531–43
24. Welch RA, Hull R, Falkow S. 1983. Molecular cloning and physical characterization of a chromosomal hemolysin from *Escherichia coli*. *Infect. Immun*. 42:178–86
25. Wolstenholme GEW, O'Connor M, eds. 1963. *Ciba Foundation Symposium on Bacterial Episomes and Plasmids*. London: Churchill