Farewell Stan

Stanley Falkow: 1934-2018

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Stanley Falkow was a giant among microbiologists, a pioneer of microbial pathogenesis research, a wonderful mentor, father of an enormous family of world leading microbiology scholars, a gifted and dedicated teacher, and a warm, friendly, generous, relaxed, articulate and humorous communicator. He will be sorely missed. Below are some fond personal memories of times and experiences of colleagues from the early days of Stan's amazing career.

Felipe Cabello

Before I graduated from medical school in Chile, one of my professors recommended I read a review authored by T. Watanabe, "Infective heredity of multiple drug resistance in bacteria," *Bacteriol. Rev.* 1963. 27: 87-115. This review excited me greatly and opened up my mind to plasmids and antimicrobial resistance. My next step was to find out more about episomes and plasmids as molecular entities. In the course of my examination of the literature, I encountered the classical papers of Dr. Stanley Falkow. He and his collaborators, noticing the difference in GC content between the chromosome and plasmids of *Escherichia coli* and *Salmonella* and bacteria such as *Proteus* and *Serratia*, used cesium chloride ultracentrifugation for the molecular determination of the extrachromosomal nature of these plasmids (see Falkow et al. Episomic transfer between *Salmonella typhosa* and *Serratia marcescens*. *Genetics*. 1961. 46: 703-6.). I found this work extremely original, motivating, and path breaking, and stimulated me to learn more about these interesting biological entities. I successfully applied for a post-doctoral position in the laboratory of Stanley N. Cohen Lab at Stanford and I have been around plasmids ever since.

I never thought that a few years after reading Dr. Falkow's papers I would meet him in person but that was indeed the case. I met him in New Orleans in 1974, and had the pleasure to interact with him many times after and appreciate his scientific insights and

bonhomie. Even if I did not work with him, as a result of a collaboration between K. Timmis, myself and Jorge Crosa, I visited his lab in Seattle a few times. Later, Stanley played an important contribution to starting my career in microbiology because he advised me, patiently and generously, on how to rewrite a previously rejected NIH grant proposal to study the role of Col V plasmids in extraintestinal infections of *E. coli*. Stanley, in several phone conversations after I mailed him a copy of my proposal, kindly showed me the lack of focus and of relevant details in the proposal and nudged me to introduce the changes necessary to correct these shortcomings. The resubmission was funded and I started my independent life as an investigator in microbiology. Thanks Stan for your time, big-heartedness and forbearance with an unexperienced and apprehensive immigrant.

Stanley N. Cohen

Stanley has written that as a young student, his reading of Paul de Kruiff's classic book, *The Microbe Hunters*~ which recounts the discoveries of van Leeuwenhoek, Pasteur, Koch, and other historically-great microbiologists ~ motivated him towards a career in microbiology. He undoubtedly could not have imagined at the time that almost two thirds of a century later, his own discoveries about how microbes cause disease and become resistant to antibiotics would have gained him a place among the giants of this field. But Stanley Falkow was not only an extraordinary scientist. He was an admired and beloved teacher and mentor whose creativity, candor, knowledge, integrity, and perspective were widely sought by colleagues, and a participant in multiple scientific advances that occurred during his lifetime. He was a voice for scientific reason at occasions where such a voice was urgently needed. He had wit, humility, kindness, and fairness; if I had to choose a single word to best describe Stanley Falkow, it is that he was a *mench*.

As a Brown University graduate student, Falkow found that the transfer of resistance genes among bacteria was associated with transfer of particular species of extrachromosomal DNA. The approaches that he and his colleagues pioneered established parameters for almost a decade of further work by multiple labs. When Stanley and I first met in 1966, I was a postdoctoral fellow seeking plasmid strains to begin work on transmissible antibiotic resistance; by generously providing materials together with much needed sage advice, Stanley helped to kick-start my own studies of antibiotic resistance plasmids~ and there began our more than 50 years of friendship and professional interaction. At our first encounter, Stanley shared with me his intent to stop doing research on resistance plasmids in favor of a move to studies of bacterial pathogenesis. This revelation generated more than a little anxiety: should I, as a neophyte, be entering a field that an acknowledged leader was leaving? But in fact, Stanley continued to contribute toward a fundamental understanding of plasmid biology for another dozen years while concurrently proceeding to provide a foundation for study of pathogenesis.

Stanley Falkow's scientific contributions are a matter of record, and there is no need to recite them here. But I'd like to share a couple of additional personal memories about Stanley, who was a lifelong and very dear friend, confidant, and colleague. From our first

encounter, and long before Stanley's move to Stanford, we talked regularly about science--often in lengthy phone calls. Our careers interacted at so many levels and in so many venues that at scientific and committee meetings our colleagues often had to distinguish which "Stanley" they were referring to. Mostly, he was Stanley and I was Stan. When I was asked to co-organize a scientific meeting on plasmids in Honolulu Hawaii in 1972, Stanley was obviously the first person on the invite list. In Hawaii, he was present at the Waikiki beach delicatessen discussion--over corned beef and pastrami sandwiches and very cold beer--that led to my collaboration with Herb Boyer on DNA cloning experiments. In fact, as a group of five of us walked along a Honolulu street seeking a place to have a late-evening snack, it was Stanley who noticed the word "Shalom" emblazoned in neon lights in the window of what we were to discover was a kosher deli operated by a Korean proprietor--and he quickly volunteered, "well....., it can't be bad".

Stanley and I were part of a small group of plasmid biologists that met together over a multi-year period to develop a nomenclature for describing plasmids. The group later morphed into a committee that provided a framework for research guidelines during the biohazard controversy about DNA cloning. Those were very contentious times, and Stanley had a crucial role in bringing consensus to the process. The worldwide respect for Stanley among people holding widely disparate views was paramount in creating guideless applicable to new methods of studying genes. When some others lacking knowledge of microbiology or bacterial disease were speculating about disasters that they claimed would assuredly come from genetically altering *E. coli* by other than "natural" methods, Stanley was intent on focusing on data. Scientific truths should have been obvious to all, but Stanley's credibility was crucial to their discovery in that climate.

Sadly, the world has lost an extraordinary scientist and human being.

Roy Curtiss III

Stan was and is a Giant. We grew up five miles apart in up-state New York, but only met when we were both 30 when he introduced my talk at the annual ASM meeting. Research on bacterial sex and especially plasmids conveying multiple drug resistance were bonding fixations for us both. Experiences in Honolulu at US-Japan meetings, Puerto Vallarta to hash out plasmid nomenclature (where Richard Novick, Roy Clowes, Stanley Cohen and Naomi Datta succumbed to enteric infections while Stan, because he had probably contracted them all and was immune, did just fine), Asilomar and multiple RAC meetings thereafter are most memorable. One of the best was sharing a room at the La Valencia in La Jolla and a bottle of Jack Daniels to hash out and write substantial portions of the recombinant guidelines until 5 am to be presented to the entire Committee that morning at 8, with complete sobriety, of course. Five years of shared science exchanges during extended weekend excursions, compliments of Bristol Myers Squibb, at Kennebunkport, the Grand Tetons, Rancho Valencia in Rancho Santa Fe and the Inn at Blackberry Farm in the Smokey Mountains will always be remembered but Stanley never induced me to be a fly fisherman. He was an expert. My last face-to-face interaction occurred at the Bugs as Drugs Colloquium in San Diego a few years ago

where we toasted each other with martinis when we were not hypothesizing science breakthroughs. As a mentor of us all, he is greatly missed.

Gordon Dougan

Stanley Falkow, who recently passed away in Palo Alto, California, can arguably be considered the leading microbiologist of his era. He pioneered the field of molecular cellular microbiology and mentored a whole generation of talented individuals to take advantage of it. He won many accolades including a Congressional Medal of Honour, The Lasker and Robert Koch Prizes and was elected to both the US National Academy of Science and the Royal Society. He was a true giant in his field who was a hugely popular scientist capable of communicating his work and beliefs broadly. He was also great company with a wonderful sense of humour and an ability to create a warm environment where ever he was.

Stanley was trained as a medical microbiologist at Brown University. He was awarded a PhD during which he developed an encyclopaedic knowledge of clinical microbiology, learning how to handle the classic bacterial pathogens, information that he exploited to great effect later in his career. In the 1960's he moved to Washington and started to exploit the emerging microbial genetic technologies to investigate antibiotic resistance. He defined the molecular nature of R Factors, the plasmids that encode multiple antibiotic resistance and helped identify transposable elements. He wrote an influential book entitled 'Infectious Antibiotic Resistance' that was prophetic about how the misuse of antibiotics would drive the emergence of resistance, greatly compromising clinical practice.

By the early 1970s he had moved his laboratory to the University of Washington in Seattle. Due to his expansive knowledge of plasmid biology he was approached early on by the pioneers of gene cloning to develop plasmid-based vectors suitable for cloning genes. He attended the famous Asilomar Conference that drew up guidelines for genetic engineering where he saw the potential of cloning in medical microbiology. Starting with enterotoxigenic *Escherichia coli*, through the cloning of the toxins and adhesins from this pathotype, he began to exploit molecular and cell biology to dissect the molecular basis of infection in multiple pathogens ranging from *Bordetella pertussis* to *Salmonella*. Some of his early work in this area, such as cloning the 'invasin' gene from *Yersinia* into *E. coli*, transformed our understanding of the biology of infection. He also performed some of the very earliest work exploiting DNA probes to identify pathogens through a project on enterotoxigenic *E. coli* infected patients in Bangladesh.

During this period Stanley moved his laboratory to Stanford University, where, as Head of Department, he assembled a world leading infection and immunity faculty. In the latter period of his career many of his trainees became world leading scientists in their own right, forming the extended 'Falkow' family. He was married to Lucy Tompkins, a renowned clinical microbiologist based at Stanford University Hospital. He continued to teach at Stanford right up until last year but spent more of his time fly fishing and

enjoying life moving between his homes in Palo Alto and Montana. The passing of this great scientist will leave a big hole in his community.

GD first met Stanley during a conference in Lunteren, Holland in 1975. After a few beers GD had secured a position in Stanley's laboratory. The two interacted and exchanged visits from then on until shortly before Stanley died. Stanley loved nothing better than chatting with young scientists, telling them stories about his old scientific heroes and collaborators such as H. Williams-Smith, Harry Smith and Naomi Datta. Anyone involved in these conversations, which were often accompanied by a glass of fine malt whiskey, came out a great deal more knowledgeable about science and life in general than when they went in.

Jan van Embden

During the fall of 1971 I learned to know Stan Falkow at Georgetown University in his narrow lab with just 2 researchers, Don Le Blanc and Patty Guerry. Unexperienced with experimental work and coming from Holland I was highly surprised how Stan worked: he was either talking in the telephone, writing together with the secretary or hanging around the scintillation counter or the analytical ultracentrifuge with Don, Patty or me to speculate about promising experimental results and all issues of life and science. In those few months I learned more than during all my previous work and he remained a main source of inspiration later on. I am very thankful to have worked together with this brilliant, humorous and warm scientist. So long Stan.

B. Brett Finlay

I had the great fortune to train in Stan Falkow's laboratory twice. The first time (1982) I was a very young 2nd year PhD student in Biochemistry at the University of Alberta. At that time, recombinant DNA was just coming of age, and the Falkow lab had pioneered many of the techniques, including restriction enzymes, transposon mutagenesis, and plasmid isolation. I went there to use these techniques to characterize an F-like conjugative plasmid pED208. Stan welcomed me to the lab as one of his own, and paired me with Dan Portnoy, who was a senior graduate student (and a wonderful tutor). The lab members read like a who's who in bacterial pathogenesis. In addition to Dan, Mike Koomey, Alison Weiss, Rod Welch, Staffan Normark and many others were using recombinant DNA to unravel mechanisms of bacterial pathogenesis and virulence mechanisms. It was heady times, with new discoveries seemingly every day. Stan oversaw this talented group, encouraging everyone on, always digging into the projects and providing valuable wisdom, often in the form of very sarcastic yet humorous comments. He had a wonderful knack of seeing the big picture, and how these various findings fit into disease. Dan helped me characterize the plasmid, and we had a lovely publication where we mapped the various functions of the plasmid, including the transfer region, the origin of transfer, where the *lac* operon was, and other functions (Finlay BB, Paranchych W, Falkow S. 1983. Characterization of conjugative plasmid EDP208. *J Bacteriol.* **156**:

230-235). Ironically, Stan had actually worked on the same plasmid in his first years with Samuel Forman at Walter Reed Army Institute of Research. They were asked to work on a *lac⁺ S. typhi* strain, fearing that it was a genetically engineered strain coming out of Russia (which of course was wrong- it was just *S. typhi* that had acquired the plasmid). For whatever reason, Stan was pleased with my work, and asked me to return when I completed my PhD as a post-doctoral fellow in his lab.

Taking Stan up on his offer, I went to his lab for my post-doctoral training (1986-1989). These were even headier years for the pathogenesis field. Recombinant DNA techniques were more easily applied to many pathogens, allowing their virulence factors to be characterized. Ralph Isberg had just cloned the invasin gene from *Yersinia*, and Stan (and everyone else) were very excited about studying how pathogens actually entered into mammalian cells. Virginia Miller was working on *Salmonella* invasion, and many others in the lab were studying a virtual menagerie of invasive bacterial pathogens. I began to study *Salmonella* uptake into polarized epithelial cells, which only recently had been characterized. This opened up the fascinating concept of studying how pathogens actually penetrated through epithelial barriers. During my post-doc, I was able to study many aspects of how *Salmonella* invaded and also penetrated epithelial barriers (Finlay BB, Gumbiner B, Falkow S. 1988. Penetration of *Salmonella* through a polarized Madin Darby canine kidney epithelial cell monolayer. *J Cell Biol.* **107**: 221-230). Stan's enthusiasm was constant, his encyclopedic knowledge amazing (and amazingly useful), and he was a wonderful mentor. His attitude was to provide a strong laboratory environment, and, in Stan's words, "let you strut your stuff". He has mentored an entire generation of scientists that, over the past few decades, have provided so many seminal findings in bacterial pathogenesis. His biggest legacy is these wonderful scientists around the world that continue this work. Stan was very encouraging and supportive with his trainees, and his humor everpresent. I will always remember his parting line when he was leaving to Montana for the summer: "work hard and make me a legend". The second half of this statement has certainly come true.

1987

Fred Heffron

No one did more for my career than Stanley Falkow. What I will always remember is his kindness and ability to get along with everyone. He was charming and charismatic. Just the type of person you wanted to be around.

I joined his lab in '73. I was not pleasant in his lab (and still not) but he was kind to me despite this. Stanley gave us Yiddish language lessons but I am still not sure whether I am a *schlemiel* or a *schlimazel*.

It is easy to forget that Stanley was not always on top of the world – he was not appreciated at Georgetown and had a grant refused when he first started at U of Washington. Because of that Stan was debating on going to medical school but fortunately for science he did not. Stanley decided to concentrate on bacterial pathogenesis because had been a med tech and inhaled a lot of medical knowledge before starting graduate school. Knowledge of medicine was put to good use throughout his career.

Some examples of his personality - at University of Washington we were in the same building as the hospital but a long ways from the clinics. Patients would saunter down the hall and ask for directions, not always nicely, at all times of day. All the rest of us barely put up with this but Stanley was always courteous and polite while we were thinking “Stan they are wasting your time”.

Stan emphasized how important it was to be friends with other scientists and to exchange scientific ideas. One example was with a well-known scientist in England who had sent several plasmids that were related but encoded different antibiotic resistance. What was exciting was that the difference in antibiotic resistance resulted from insertion of DNA into the same DNA backbone. His friend in England published the best picture showing this, which she had obtained from Stan, but that meant that meant Stan could not publish it. Yet the two remained close friends.

Stanley's excitement about science, his kindness and his generosity were lessons we all carried with us. I will miss him.

Don Helinski

Stanley Falkow was a pioneer in the development and employment of molecular and genetic tools for the elucidation of the molecular basis of bacterial pathogenicity and the genetic basis of antibiotic resistance in bacteria. His discoveries provided the foundation for much of the later work in these two critically important areas. Through his research and insightful reviews of published work in these areas of research, he had a profound

influence on the development of the fields of bacterial pathogenicity and the genetic basis and dissemination of bacterial resistance.

His loss will be deeply felt by his students, postdoctoral fellows, and colleagues.

Richard Hull and Sheila Hull

We worked in Stanley's lab as post-doctoral trainees in the late 1970's. Stanley's pioneering research contributions to molecular mechanisms of pathogenesis are well known. His vision opened many new areas of research.

However to us, his most enduring legacy to biomedical research is in his superb skill and commitment to training of graduate students and post-doctoral fellows. He provided an environment that supported intellectual growth. His guidance instilled skills necessary for success as biomedical investigators as well as conveyed his passion for independent/creative thinking.

1979

Ralph Isberg

I first learned of Stanley Falkow when I was a 20 year-old student taking college Microbiology. My professor had Falkow's "Infectious Multiple Drug Resistance" on his desk, received as a gift from the author. Opening the book I saw a distinguished looking gray-haired scientist, appearing very much the sage. After spending some time gazing at the photo, my professor said, "Don't trust the gray hair. He is only 41 . . . and never misses an opportunity to make a scatological reference!" At the time, 41 appeared

sufficiently ancient to rank as a sage, so I didn't know how to reconcile the scatological tendencies with the photo.

This paradox lingered with me several years until, as a more sophisticated senior graduate student, I travelled across Boston to hear my first Falkow lecture. He spoke excitedly about studying *E. coli*: "You know, you really can learn a lot from growing bugs in pee." Truthfully, pee had never been my cup of tea, nor was diarrhea, another favorite didactic dish he served up at the lecture. What my college professor said appeared to be true. Now I had to deal with the fact that my Plan A was to do postdoctoral work with Falkow (I had no Plan B for my future), and poop held a fascination for him that I didn't totally understand. Although our shared ethnic heritage resulted in tortured intestines, clearly some of our brethren embrace this weakness, some run away from it, while the rest become gastroenterologists.

Plan A started shortly after quickly glancing at the book, when a class assignment asked us to present exciting findings in microbiology not found in our textbook. Two months before the assignment, the Heffron, Rubens and Falkow paper had been published, which showed that an ampicillin resistance cassette had the ability to translocate from one DNA site to another (Heffron F, Rubens C, Falkow S. 1975. Translocation of a plasmid DNA sequence which mediates ampicillin resistance: molecular nature and specificity of insertion. *Proc Natl Acad Sci U S A* 72: 3623-3627.). They called it a "jumping gene" at the time, and no one in the class had ever heard of this phenomenon. Gene translocation would become the focus of my Ph.D. thesis, but I did not forget the paper and the scientist who led me to this field. The problem now was that he was no longer interested in this stuff, and microbial pathogenesis had become his research focus. Somehow, he had moved from mapping the ends of DNA insertions by electron microscopy to growing *E. coli* in pee. If I believed that he was a sage, I had to believe that he could move the field in the direction that he wanted it to go. So, here comes the confession shared by many in the field: we started working on microbial pathogenesis *because that is what Stanley did*. It was that simple.

When Stanley accepted me into his lab as a postdoc, he never actually said yes, he just asked me about my plans for a fellowship proposal. After a little studying, I said I wanted to work on how bacteria get into nonphagocytic cells. He told me it would never get funded, just write about work related to gaps in the *Yersinia* field that were preventing further progress. Of course I followed his directions, it got funded, but I really didn't want to do pursue these studies, and I arrived on campus, hoping that I would not have to work on the topic of the proposal. Arriving on the Stanford campus, my first meeting with Stanley was scheduled, at which time we would talk over projects. I was wondering: should I tell him what I want to do, or should I just listen and follow his advice?

At this point, it was time to take an important lesson from Jon Beckwith, a towering figure in microbial genetics whose lab was next to mine during my thesis research. I noted that if a new postdoctoral fellow listened to Jon, the project always worked. These people invariably went on to become leaders in their fields. Listening, therefore, did not mean lack of independence. In contrast, postdocs who failed to heed Jon's advice uniformly pursued failed projects.

The route was now clear. If you choose an advisor based on the fact that this mentor will be the most effective at promoting the progress of your career, listen and trust your instincts. Listening shows confidence in your ability to make a good career decision, it is not a sign of weakness. So, Stanley would suggest a project, and I would do it. It was settled. Perhaps on the side, if I had time, I could begin to work on determining how either *Salmonella* or enteropathogenic *Yersinia* enter mammalian cells. No matter what, this project had to be secondary to whatever he said, because he understood the field better than I did. The plan now set, I walked into his office, sat down, and exchanged some pleasantries about arriving in California. Then he said, "Why don't you clone *Yersinia* invasion [into mammalian cells]." I was so dizzy with glee, I can't remember the rest of the conversation.

As I read over these memories, they strike me as a view of Stanley Falkow from 10,000 feet, followed by closer circling leading to a clear view. This differs greatly from my recounting of a quiet discussion over a bright field microscope that pulled in Stanley's own memories (<http://blogs.plos.org/speakingofmedicine/2018/05/11/stanley-falkow/>). This emphasizes that my memories of him include my intimate associations with him, but also intensely involve the thoughts of a young student who only knew him by reputation. It is important for those who have trained in the period since Stanley retired, and not directly been associated with him, to understand that his great impact extended beyond his lab to very young scientists just becoming exposed to the world of research. So great was this impact, that workers entered the field of microbial pathogenesis for the sole reason that it gave them the opportunity to be trained by Stanley Falkow.

Dennis Kopecko

Dr. Stanley Falkow trained as a classical bacteriologist involved in bacterial taxonomy (the target of bacterial research at that time), to which he applied evolving bacterial genetic techniques to better understand evolutionary relatedness (well before DNA sequencing was envisioned). Following the Japanese discovery of transmissible antibiotic resistance in medically important bacteria, Stan quickly associated this phenomenon with the presence of transmissible extrachromosomal elements (bacterial plasmids). While studying antibiotic resistance genes/plasmids, Stan and others discovered that many important bacterial virulence/survival/metabolic determinants were also encoded on plasmids. Through the application of improved biochemical, genetic (cloning), immunological, and microscopic visualization techniques, Stan helped open the era of molecular microbiology and its application to modern medical and environmental bacteriology.

Perhaps most instructive to current students in molecular microbiology, Stan struggled with anxiety at public speaking for much of his career. However, he overcame this issue and was considered a singularly fantastic lecturer, enjoyed not only for his science, but also for his spontaneous humor and the ability to fit existing data into a biologically understandable story.

Dr. Falkow has been a major contributor to our molecular understanding of how organisms interact and survive in the environment, as well their ability to cause disease. His wit, intelligence, and voluminous publications will long be appreciated!

Stuart Levy

There has been an extraordinary advancement of scientific knowledge through Dr. Stanley Falkow's work. From my standpoint, his work has also touched us in the field of antibiotic resistance. Stan was a wonderful colleague and mentor. His passing has left us with a great legacy of scientific excellence. I join a great many in our scientific community who mourn his loss.

John Mekalanos

I first met Stanley Falkow at a Toxins Gordon Conference about 40 years ago. I was John Collier's graduate student at the time and was selected to give a short talk in front of some tough, critical scientists ~ Michael Gill, Dick Finkelstein, Sam Formal, Harry Smith, to name a few. I was terrified. After the session ended Stan sought me out and told me "Don't worry about those guys, keep doing what you are doing and hang loose!" It was a seminal moment for me to be noticed by Stan. I had heard a lot about him from Ralph Martinez at UCLA who featured Stan's work in lectures to us as undergraduates (Steve Lory, Dan Portnoy, Michael Thomashow, to name a few). Stan had that kind of reputation and influence already. I eventually sought Stanley out as a collaborator when I was a postdoc in Jack Murphy's lab at Harvard. While working with Steve Moseley in the Falkow Seattle lab, I remember accidentally breaking an expensive UV light box and how Stan simply said "S~ happens" and refused to let me pay for it out of pocket when I offered. Of course Stan's scientific generosity is legendary but his personal warmth, humor, and scientific acumen was a combination that I had never quite experienced before. He was such an awesome role model at a critical stage of my career. He was and became a giant in our field over, and over, and over again! I also became very close with his numerous trainees through the years. Friends and colleagues I will value all my life. Stanley's impact on our field cannot be easily measured because the Falkow extended family is so large and accomplished. It will continue to grow and achieve for years to come. As Stan used to say "Making me more famous."

Incidentally, I only saw Stanley mad once. At a US-Japan cholera conference he became furious when a prominent investigator in the cholera field challenged him openly on the conclusion that the heat-labile enterotoxin of *E. coli* was basically cholera toxin ~Do you expect us to believe THAT? the critic said. The evidence ~ Walt Dallas had cloned the LT genes in Stan lab and analyzed them by mini-cell expression analysis and product immunoprecipitation. The data was so clear! After that confrontation, Stan told me "Johnny, I'm out of here ~ I'll never come to this conference again!" And he never returned. Of course Stanley was right, but the field was only beginning to understand the types of molecular analysis of pathogenicity factors that his group was pioneering.

A decade later, just the two of us travelled to Taiwan to give special lectures. What a great experience! Stan shared numerous scientific insights and a lot of advice with me during sightseeing trips and the occasional bar stop. For example, he told me that

arrogance does not serve young scientists well and that I should try to conduct myself in a humble manner even when I had 'the goods' and the other guy was just plain wrong. I took that to heart because I knew Stanley would only bring up such a personal and sensitive topic if he knew it would help me. I think that advice made a huge difference in my success as a scientist and I pass it on to my trainees when it seems appropriate. Stan also offered many funny life lessons to me during that Taiwan trip. On one particularly long drive he told me.... "Johnny as I got older in life I've learned three things - Never miss an opportunity to _____, never trust a _____, and never waste a _____". For those of you that know Stan well, you'll likely laugh guessing what fills in those blanks! Hint: bodily functions of course! I will deeply miss sharing single malts with him and talking about anything he was interested in - from fishing, to flying, to fecal transplants. Warm memories for sure. I miss you Stanley.

Richard Novick

I can't remember when, in the mists of time, I first met Stanley, but it would have to have been sometime in the mid 60's - soon after his seminal paper with Julius Marmur demonstrating plasmid DNA, and followed by our long and parallel careers on plasmid biology and its impact on antibiotic resistance, with Stanley diverging to found the field of microbial pathogenesis; but not before a memorable evening in Honolulu (1971, I think) when Stan Falkow, Stan Cohen myself and others were having a drink at the bar and discussing the power of the recently discovered restriction enzymes. As I recall, it was Stan Falkow who first realized that the sticky ends of *EcoR*I-cut DNA from different sources might be pasted together to form a hybrid molecule - the birth of "recombinant DNA", as it came to be called.

Stan and I only ever co-authored one paper - a proposal on the nomenclature of bacterial plasmids.

Random vignettes:

- * A meeting - must have been in the mid sixties - in which I was sitting next to Stan to hear a tremendously exciting talk by Marshall Nirenberg describing his decipherment of the genetic code. His talk was followed by Severo Ochoa, who had really missed the boat - we were amused to see Ochoa one-upped, despite our (or at least my) tremendous respect for Severo.
- * Many wonderful Plasmid Biology meetings where we were always comparing his work on *Salmonella* with mine on *Staphylococcus*, though, as I said, he became more and more interested in pathogenesis while I went more and more deeply into plasmid biology.
- * An excited conversation with Stan - early 90's - in which he described his beginning to use GFP and I did not see, at the time, what was so special about it.
- * My last meeting with Stan was in Stanford - perhaps 2013 - to organize a conference on the history of plasmids, to be held at the Banbury Center of Cold Spring Harbor in 2014. Stan had already been suffering for several years from myeloid dysplasia, had adamantly refused to have a bone marrow transplant and was doing extraordinarily well despite it; but he had had a mild stroke and was afraid he would not be able to travel NY

to present his paper. As it happened, he sent a video of himself presenting it, which went so well that many were wondering why he hadn't come after all. It was all a bit sad, because he would so greatly have enjoyed seeing all the old-timers who showed up.

José Miguel Ortiz Melón

Around 1972 Stanley's lab in Seattle was small and friendly. I remember we were only six people: Jorge Crosa and myself, as postdocs, Maggie So and Fred Heffron, as PhD students, and Patty and Caryl-Sue as Stanley's lab technician and secretary. Stanley was most of the time in his office reading and writing and we used to come into it freely to ask him for advice, report results and to comment about all kind of small problems that may arise. He was prepared to listen to anyone and to help as long as he could do something about it.

At that time plasmid scientific leaders were also a small group namely Stanley Cohen, Don Helinski, Roy Clowes, Roy Curtis, Naomi Datta and Richard Novick. They used to have restricted scientific meetings in touristic places, usually in hotels. Although I did not attend any of these meetings, I believe that beside more or less formal presentations it was common to talk about progress of their labs around the bar and, although there were all good friends, there was a fair amount of rivalry among them.

At that time I was struggling trying to study replication of different plasmids in *E.coli* dna mutants, and the results were not clear. Other people in the lab were also starting their projects and did not have many things to report. Stanley was due to attend one of these restricted meetings in a small place in Mexico. It was known that he was nervous about flying in what he called *Mickey Mouse* airlines. Nevertheless, he had registered for the meeting and everybody thought that he would go. Therefore, it very surprising for some of us to see him earlier than usual in his office while we thought he will be off. When asked about it he was not very clear about it and I never knew whether he had cancelled it because he did not have much to report or because of fear of flying in dodgy planes.

1973

Stanley and Siena. The first time I met Stanley was a long time ago at a big meeting in building 10 of the NIH. I had a short 10-minute talk about the diphtheria toxin mutant CRM197 immediately after the long talk of Stanley. I was absolutely sure that nobody would be interested in my talk after the famous Prof. Falkow. I rehearsed my talk many times to make sure that at least I would be able to do it within the 10 minutes and I would not leave a bad memory. In spite of my modest expectations, I received some good feedback for my talk and few years later I learned that even Stanley could remember it. My second meeting with him was in Umea, Sweden in 1983 at a course organized by Staffan Normark. He gave a talk on pertussis and I can remember even now how well he could mimic the paroxysmic cough and explain how the bacterium caused the disease. He also described work done in his lab by Alison Weiss and the isolation of transposon mutants affecting some of the virulence factors. I did not have the opportunity to talk to him in Umea, but that talk convinced me that making a better pertussis vaccine was going to be my next project. A few months later, I asked my friend Gary Schoolnick at Stanford whether he could introduce me to Stanley and organize a meeting with him to learn more about his work on pertussis. This was the first time I could introduce myself to Stanley and we could talk. Stanley was very nice, he walked me through the work on pertussis in his lab, showed the latest data and gave me the mutants of Alison Weiss. Those mutants allowed me to enter the pertussis field, to clone the operon coding for pertussis toxin, to clone the gene coding for FHA, and finally to make a vaccine. In the meantime, we had become good friends and I familiarized with the people in his lab so much that Scott Stibitz provided me with the plasmid to delete and insert genes into *Bordetella pertussis*, and David Relman came to Siena to sequence the gene for FHA. When in the early nineties I established a scientific advisory board for Chiron Vaccines, I asked Stanley to be part of it. Being on the SAB provided for him the opportunity to come often to Siena, where he became a friend of many young scientists with whom he would spend a long time listening and advising. Some of them, like Antonello Covacci, Stefano Censini and Fabio Bagnoli, spent time in his lab at Stanford; others, like Mariagrazia Pizza, just became very close friends. He asked me how they were doing when I met him last time in January 2018. The picture, although of not great quality, is how I remember Stanley in Siena: walking among the olive trees that surround the Research Center, talking and motivating young scientists.

1996 or 1998

Marilyn C. Roberts

During my graduate study in Stanley's laboratory at the University of Washington he did not travel. He had a modest number of post-doctoral fellows ($n=3$), a Research Associate and 5-6 graduate students when I came into his laboratory. There were a few main projects and I was given the project to look at the mechanism of penicillin resistance in *Neisseria gonorrhoeae* isolates coming from Asia which had just appeared. The news media was in hysteria over this bacteria because our troops were coming home with this pathogen that was totally resistant to penicillins, the first therapy of choice.

Previously in the laboratory the group had found a simple process to lyse bacteria and run it on an agarose gel to look for plasmids. Stanley predicted correctly that the resistance to penicillins in *N. gonorrhoeae* would be a *bla*_{TEM} on a plasmid just like was recently identified in ampicillin resistant *H. influenzae* species. He was correct about the *bla*_{TEM} but this time it was associated with a gonococcal plasmid that was mobilizable but not self-conjugative. My thesis work took off and I was an author on 6 publications [5 first author] and another 6 book chapters. This included the paradigm changing conjugation experiment paper published in Nature [Nature (London) 266:630-631, 1977. PMID 404570] which showed for the first time that bacteria other than *E. coli* and related genera carried conjugative plasmids with antibiotic resistance genes that could be moved by conjugal transfer.

We always had famous or soon to be famous people visiting the laboratory for a short or long term. This allowed graduate students the ability to interact with top scientists in the field and develop connections and long term friendships. Stanley inspired us to work hard but there was not competition as much as collaboration among students. We worked long hours but enjoyed every minute of our time. Many of the skills I learned first hand from Stanley as a graduate student stayed with me and defined the researcher I became.

He also was very accessible and inspired us to work at a high level and in my case 7 days/week. I can remember Lynn Elwell PhD telling me to call Stanley on a weekend because we were so excited about our first mating experiments and I had transferred the β -lactamase between *N. gonorrhoeae* isolates. He was as excited about the results as we

were. This began my career long interest in antibiotic resistance genes and how they move between bacteria and within the ecosystems.

Stanley went on a sabbatical leave in the summer of 1978 in the UK. I can remember Stanley writing me during the summer and saying you have enough data just put some of your many publications together, write an introduction and a conclusion and call it your thesis that way you should be able to finish by Fall quarter. That was the first time anyone in the department had done this for their PhD thesis. This became the standard for my students to do the same thing.

Stanley was a great writer and improved my scientific writing skills. He also was a fabulous seminar speaker and lecturer. He could entertain and educate medical students like no one else could.

Magdalene So

Stanley Falkow was a luminary in the field of infectious diseases and microbial pathogenesis. He pioneered the use of molecular biology and genetics to understand the mechanisms by which bacterial pathogens cause disease. During my graduate days in his lab at the University of Washington, Stanley discovered transposable elements and pioneered its use as a mutagenesis tool (Fred Heffron worked on that; he published on Tn3, at the same time that Nancy Kleckner published on Tn10 and Doug Berg on Tn5). He published seminal papers on plasmid replication (Jorge Crosa). He found that virulence genes could be transferred between bacteria via plasmids (yours truly).

Stanley imparted his excitement of scientific discovery to all of us; we couldn't wait to get into the lab in the morning to look at our plates. He closely monitored our daily progress. He always arrived at work early and by the time I staggered in he would have read my plates and would greet me by telling me about my results. I didn't like that because I wanted the thrill of it first-hand.

Molecular cloning was just getting under way at that time, and there was a lot of concern that recombinant DNA would pose a threat to society. Stanley played a huge role in convincing scientists that ordinary lab *E. coli* harboring recombinant DNA would not be the Fifth Horseman of the Apocalypse. One day I found Stanley in his office surrounded by stacks of old microbiology textbooks. When asked what he was doing, he said he had just returned from a meeting (the first ever) on molecular cloning, and was asked by its newly formed recombinant DNA committee to find out how many bacteria lived in the gut of cockroaches and whether lab *E. coli* would survive there.

Stanley took science seriously, though not necessarily scientists or administrators. He was charming and irreverent, as occasion demanded. He had the gift of gab, and was entertaining no matter the subject. I could listen to him for hours.

I owe my career to Stanley. At the end of my first year in graduate school, I received poor evaluations on my rotations and was on the verge of being dropped from the program. Stanley took up his new position in the department at that time, and after a conversation with the department chair, John Sherris, took me into his lab and gave me a second

chance. From this one instance, I learned to give my own students a second chance. Oh, he also taught me a lot of Yiddish.

Ken Timmis

Felipe Cabello and I postdoc'd with Stanley Cohen from 1973-6, studying plasmid replication, and especially its control. Plasmid biology, antibiotic resistance and transposons/transposition were hot topics in those days and frontiers were aggressively pushed forward, especially in the three heavyweight West Coast labs of Don Helinski, Stan Cohen and Stan Falkow, that seemed to publish only in *Nature*, *Science* and *PNAS*. Reflecting the excitement, plasmid meetings were organised regularly and, since the plasmid-transposon community was reasonably small and friendly, if keenly competitive, everyone knew more or less everyone else and happily exchanged information and materials. For a European in those days, i.e. before the advent of EC-funded collaborative research, this openness and generosity of exchange was an incredible eye-opener and, for many of us, life-changing in the way we would conduct research when we returned to Europe.

At one such meeting, Felipe met Jorge Crosa, sadly also now departed for that generously-funded research centre in the sky, who was a postdoc of Stan's and they discussed latest results. We and, as it turned out, Jorge, had repeatedly spotted a small shoulder spike on peaks of heavily radioactively-labelled supercoiled plasmid bands resolved on CsCl-ethidium bromide gradients that, when visualised by electron microscopy, turned out to be circular plasmid molecules of low-zero superhelix density which we proposed were newly-replicated daughters awaiting gyrase-mediated supercoiling. This encounter led to frequent stimulating exchanges, back-to-back publications of the story (Timmis, K., Cabello, F. and Cohen, S.N. 1976. Covalently closed circular DNA molecules of low superhelix density as intermediate forms in plasmid replication. *Nature* **261**: 512-6; Crosa, J.H., Luttrupp, L.K. and Falkow, S. 1976. Covalently closed circular DNA molecules deficient in superhelical density as intermediates in plasmid life cycle. *Nature* **261**: 516-9), and nice subsequent interactions with Jorge and Stan.

But, as our interests diverged and became less plasmid-centric, with Stan becoming increasingly focused on bacterial pathogenesis mechanisms, and me becoming increasingly focused on environmental microbiology, we saw less and less, though kept track, of one another. The last time we had contact was at the end of 2016 when I wrote to ask him if his quote that *sex was simply the mixing and matching of mucus membranes (which had in recent years become more and more innovative)*, which I think he first articulated in an ASM plasmid meeting in Santo Domingo (in 1981?), and the associated illustrative stick cartoon,

were published anywhere, since I wished to cite them in a piece of educational humour I was writing. He was very happy to reconnect and told me that he still taught medical students 3-4 h per week, had drawn the cartoon in the middle of one of his early lectures on mechanisms of transmission of infectious diseases, and still used the phrase and cartoon in lectures and seminars. But no, they had never been published and he would be happy for me to do so. Sadly, he did not live to see them in print. Anyway, my first - unanticipated - use of them is here; the planned use will appear later in the year. The other thing we discussed was T. W. Burrows, the famous *Pasteurella* (*Yersinia*) *pestis* pioneer and his work at Porton Down (classical, ground-breaking research, taught enthusiastically to undergraduate microbiologists at Bristol in the mid-60s, one of whom just happened to be T. W.'s daughter, Pam), whose name I had blatantly exploited for a fictitious microbe - *Burrowsia* - I created for another educational humour piece: Stan knew Burrows and remembered well the stories about him, so was able to fill in key gaps in my knowledge.

Stan was a giant in the field of infectious diseases: through his brilliance, impressive intuition for the key to the problem, recruitment and mentoring of exceptional students and postdocs, and foresightedness in applying latest technological developments, he led all the way. His contribution to our understanding of mechanisms underlying infectious diseases is immense. And his infectious enthusiasm as a teacher and mentor have

influenced generations of medical students and young researchers. Stan was also an incredibly personable individual: exceptionally helpful to colleagues at all stages of their careers, warm, friendly, eloquent and humorous.

The last two comments in our exchanges were: *I read the obituaries every day and duly note if the subject is 'older', 'younger' or 'right on' in age, and I am going to now go and drink a whiskey while watching the snow fall at our Montana home.* It has been for me, as for so many others, a great privilege to have known, enjoyed and benefitted from Stan. He will be sorely missed. Though I do not drink whiskey, I will raise a glass of good wine to fond memories of him.

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