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Virulence profile

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When did you first become interested in science?

As I remember it was in the second grade when I learned about trilobites and dinosaurs. Although I had a terrible biology course in high school, I read a book by Ralph Buchsbaum called *Animals Without Backbones* (published in 1938) that kept me excited about biology. It had a lot of wonderful drawings. My father had a microscope that he got from his father, and as a child I used to spend hours looking at paramecia and other protists in pond water.

What was your career path that led you to where you are today?

It was a very circuitous one. I started as a biochemist in college but shifted to microbiology/molecular genetics as a graduate student and as an Assistant and Associate Professor at Harvard University. When I moved to Harvard Medical School in 1983, I became a plant geneticist, and then in the 1990s I returned to microbiology. Finally, in the late 1990s, the lab became focused on studying host–microbe interactions using *Arabidopsis thaliana* and *Caenorhabditis elegans* as model genetic hosts.

How did you become a microbiologist?

My first major at the University of Illinois at Urbana in 1962 was engineering physics, but that only lasted a few weeks because I discovered that I did not like doing the math. I then switched to chemistry. I can't remember what motivated that decision. The chemistry department required that majors specialize in analytical chemistry, organic chemistry, physical chemistry, or biochemistry. This was initially a tough decision, but as a sophomore I had an outstanding organic chemistry professor, Douglas Applequist, who sparked an interest in biochemistry. If I was going to become a biochemist, I realized that I needed to learn some biology. Instead of taking the introductory biology course, however, I enrolled in an experimental honors biology course taught by Judy Willis, who is now at the University of Georgia and with whom I had the pleasure of reuniting with just last year. Judy Willis was also an excellent teacher, and she had a major influence on my decision to go to graduate school in biology.

But in the meantime, I was committed to being a chemistry major, and I only had room in my schedule to take a couple of biology courses. One of these was a microbiology course, but my first introduction to microbiology was not a success. On the first day of lab, I couldn't get my Bunsen burner to light (the teaching assistant pointed out that I had attached it to the vacuum line), and at the next lab I flamed my alcohol bottle and got burning alcohol all over my arm. The TA told me in exasperation that I would never be a scientist. But on a brighter note, I remember very distinctly going to a seminar given by Sol Spiegelman, a professor at the University of Illinois, at which he announced to a standing-room-only crowd that he had been able to achieve in vitro replication of infectious RNA from the bacteriophage Q-β. I was convinced. I wanted to become a microbiologist/molecular biologist.

First I had to graduate as a chemistry major. I really wanted to do a senior thesis project, and I chose the laboratory of the

biochemist/microbiologist IC Gunsalus. This turned out to be a fateful decision. "Gunny's" lab worked on a variety of catabolic enzymes synthesized by pseudomonads that allowed the bacteria to grow on many unusual substrates. My senior project was to purify and characterize borneol dehydrogenase from *Pseudomonas putida*, a perfect undergraduate project. I could do it on my own with relatively little assistance and learned how to work independently in the lab. When it came time to apply to graduate school, Gunny had many colleagues all over the US and facilitated my acceptance into the Biology Department at MIT. I arrived at MIT in 1966, the same year as a new assistant professor, Ethan Signer, who had just come from his postdoctoral training at the Medical Research Council Laboratory of Molecular Biology in Cambridge (UK) and at the Institut Pasteur in Paris, where he worked with Sydney Brenner, François Jacob, and Jacques Monod. Ethan taught a critical thinking seminar course that became legendary at MIT, and I attempted to model the way I thought about science and experiments on Ethan's approach. It is difficult to teach someone how to think critically, but Ethan was as good at it as anyone that I have known. I joined the Signer lab and starting working with bacteriophage lambda the first week I was at MIT. I was now a microbiologist.

When and how did you start working on infectious agents?

As a graduate student I worked on the phage lambda integrase protein. Lambda, of course, is an infectious virus, but as graduate student I was not interested in its role as an infectious agent. For my postdoc, I thought that I should switch to a eukaryotic system. This was 1972, and Jim Watson had declared that the golden age of prokaryotic molecular biology was over. Most of my peers switched to yeast or to mammalian systems, but I became interested in agricultural biology, mostly for political rather than scientific reasons. In 1971, my PhD advisor Ethan Signer was one of the first American scientists, along with Arthur Galston from Yale, to be invited to the People's Republic of China and returned from that trip enthused about doing "science for the people" in general and specifically about applying the tools of molecular biology and molecular genetics to agricultural science. Ethan and I realized that one aspect of agricultural science, nitrogen fixation, which limited food production, was primarily microbiological and would be relatively easy for us to start investigating. While still at MIT as an instructor teaching the Introductory Biology Laboratory, I started working on the construction of a lambda-like transducing phage that carried the nitrogen fixation (*nif*) gene cluster from *Klebsiella pneumoniae*. In 1973 as a postdoctoral fellow at the University of Sussex in Brighton with Frank Cannon and later at Harvard with Lawrence Bogorad, I switched to cloning the *nif* gene cluster using newly invented recombinant DNA technology. In the meantime, Ethan Signer started working on the *Rhizobium*–legume symbiotic nitrogen fixation system. Rhizobia elicit the formation of symbiotic nitrogen-fixing nodules on the roots of legumes in which the bacteria transform themselves into nitrogen-fixing bacteroids that are located *inside* plant cells.

I continued the *nif*-gene cloning work as an Assistant Professor at Harvard in 1975 and soon thereafter also started working on the *nif* genes from *Rhizobium meliloti* when Harry Meade, a PhD student from Ethan Signer's lab, joined my lab as a postdoctoral fellow. One of my first graduate students, Gary Ruvkun, now my colleague in the Department of Molecular Biology at MGH, had shown that *nif* genes are highly conserved, even at the DNA level, allowing us to jumpstart the work on the *Rhizobium nif* genes. My colleague Alwin Pappenheimer at Harvard was fond of calling the *Rhizobium*–legume symbiosis an infectious process. Thus, I date my work on infectious agents to ca. 1976–1977 when Harry Meade joined the lab and we started to work on the *R. melliloti*–alfalfa symbiosis.

How did you start working on pathogenesis?

When I was a postdoctoral fellow attempting to clone the *Klebsiella nif* genes, I was thinking that the long-term agronomic goal of the project was to transfer functional nitrogen fixation genes directly to plants, even though there are no eurkaryotic species that are known to fix atmospheric nitrogen. While still at MIT, I became interested in using the model plant *Arabidopsis thaliana* as a system for developing plant molecular genetics. *Arabidopsis* was touted in the literature as the *Drosophila* of the plant world, which made sense due to its small size, rapid generation time, tiny seeds, large seed set, and facile genetics. Indeed, my first NSF grant at Harvard was entitled "Transfer of Functioning Nitrogen-Fixing Genes from Bacteria to Plants". The plant was *Arabidopsis*. Although I did some work with *Arabidopsis* when I was a postdoctoral fellow in the UK, after I moved to Harvard (1975–1982), we became more and more focused on the molecular genetics of *nif* genes and their regulation and never got to the *Arabidopsis* part of the *nif* gene project.

In 1983 I moved to the newly constituted departments of Molecular Biology at MGH and Genetics at Harvard Medical School and decided that it was time to start working on *Arabidopsis* again after hearing a lecture on the power of *Arabidopsis* genetic analysis by Christopher Somerville at a Gordon Conference. Moreover, important work from Elliot Meyerowitz's lab at Cal Tech showed that *Arabidopsis* has a small genome, facilitating map-based cloning of genes identified by phenotype. Although my interest in *Arabidopsis* had been re-ignited, it was clear that the transfer of *nif* genes to plants faced too many technological problems to be practical, at least in the near term with current technology. Moreover, because *Arabidopsis* is not a legume, it does not form nitrogen-fixing nodules and cannot be used to study symbiotic nitrogen fixation. Because I was interested in continuing to work on host–microbe interactions, I decided to switch the major focus of my laboratory from symbiotic nitrogen fixation to host–pathogen interactions in *Arabidopsis* and to take advantage of the newly developing *Arabidopsis* genomic tools to identify and clone *Arabidopsis* genes involved in the host response to pathogen attack. *Arabidopsis* had not been used extensively to study microbial pathogenesis, but it was not difficult to identify a variety of previously identified bacterial pathogens that would readily infect *Arabidopsis* plants

in the laboratory. Among these was a *Pseudomonas syringae* pv *maculicola* isolate that we named *Psm* ES4326. Although my laboratory had previously worked with pathogenic microbes, including the free-living nitrogen-fixing species *K. pneumoniae*, as in the case of bacteriophage lambda, I had not been interested in the fact that *K. pneumonia* is an important pathogen in addition to being a free-living nitrogen-fixing species. Thus, work in my laboratory studying pathogenesis was initiated when the lab started working with *Psm* ES4326 in about 1985.

About Frederick M Ausubel. Dr Ausubel graduated from the University of Illinois with highest honors in 1966 with a BS in chemistry and from the Massachusetts Institute of Technology in 1972 with a PhD in Biology. He performed postdoctoral work at MIT, the University of Sussex in Brighton in the UK, and at Harvard University. He joined the Department of Cellular and Developmental Biology at Harvard as an Assistant Professor in 1975. In 1983 he joined the newly constituted departments of Genetics at Harvard Medical School and Molecular Biology at Massachusetts General Hospital as Professor of Genetics and has remained at MGH until the present time. Dr Ausubel has served on a variety of editorial boards over the years including *Journal of Bacteriology*, *Molecular Plant–Microbe Interactions*, *Annual Review of Genetics*, *Current Protocols in Molecular Biology*, *Current Biology*, *Current Opinion in Plant Biology*, *Genome Biology*, *Plant Physiology*, *Proceedings of the National Academy of Sciences USA*, *Molecular Microbiology*, *PLoS Pathogens*, *PLoS One*, *mBio*, and *Virulence*. He was elected to membership in the National Academy of Sciences in 1994, the American Academy of Microbiology in 2002, the American Association of Arts and Sciences in 2003, and the Massachusetts Academy of Sciences in 2008.

In your work on host–microbe interactions over the years, how did you decide to work on the microbe, the host, or both?

Although I was trained as a microbiologist, once I started working on symbiotic nitrogen fixation in the 1970s, I was interested in pursuing a molecular genetic analysis of the response of leguminous plants that leads to the formation of a nitrogen-fixing root nodule. However, one of the frustrations that I had working on the *R. meliloti*–alfalfa nitrogen-fixing symbiosis was that alfalfa was not at all amenable to genetic or genomic analysis, at least in the 1980s. Moreover, at that time there had not been a concerted effort in the field to develop a particular legume as a model system. On the other hand, when I decided to start working on the *P. syringae–Arabidopsis* infection model, I could take advantage of a worldwide movement that was gathering momentum to develop *Arabidopsis* as a "reference" plant for plant genetic and genomic analysis.

But what aspect of the plant defense response to focus on? It was actually a relatively straightforward decision, which was greatly influenced by the work of Brian Staskawicz at UC Berkeley, which in turn was influenced by the work of Howard Flor in the 1950s. In 1954, Flor proposed the so-called "gene-for-gene hypothesis" to explain the observation that in crop plants, resistance to a pathogen is frequently correlated with a genetically defined interaction between a plant resistance gene and a corresponding pathogen avirulence gene. This hypothesis became a cornerstone for plant breeders attempting to develop disease-resistant crop varieties. Brian Staskawicz was the first to clone a bacterial avirulence gene in the early 1980s (coincidentally using technology developed in my laboratory by Sharon Long to clone *R. melilioti* genes involved in the nodulation process). When my lab started working on the *Arabidopsis–P. syringae* infection model, the Holy Grail of the plant pathology field was to clone a corresponding plant resistance gene. Working in parallel in a friendly but cooperative competition with the Staskawicz lab, we were able to identify genetically and then clone the *Arabidopsis RPS2* gene that corresponds to the *P. syringae* avirulence gene *avrRpt2*. RPS2 (along with the tobacco *N* gene cloned by Barbara Baker's lab) was the founding member of what is now recognized as a very large family of conserved pathogen-receptor genes in plants. Genes with a similar domain structure, so called NLR (NOD-like receptor) proteins, also play a key role in the animal innate immune response.

How did your laboratory start working with *Pseudomonas aeruginosa* and *Caenorhabditis elegans* as a model pathogen and host, respectively?

The work on *P. aeruginosa* began when postdoctoral fellow Laurence Rahme joined my lab in 1991. She was previously a graduate student in the plant pathology department of UC Berkeley and was aware of experiments that had been performed over the years showing that clinical isolates of *P. aeruginosa* could infect plants. However, nobody had rigorously shown that the same *P. aeruginosa* strain was capable of infecting plants and a mammalian host under laboratory conditions. For her postdoctoral project, Laurence proposed to identify particular *P. aeruginosa* strains that could infect both plants and animals, if it were possible. Indeed, Laurence found that many *P. aeruginosa* strains were broad host-range pathogens capable of infecting both *Arabidopsis* and mice. Laurence chose one particular multi-host strain, PA14, for in-depth characterization. Subsequently, graduate student Man-Wah Tan found

that PA14 also infected and killed *C. elegans*. Importantly, we found that the same PA14 virulence factors required for pathogenesis in plants and nematodes are also required for pathogenesis in mice. With this work, the *C. elegans* pathogenesis model became an area of focus in the laboratory. Similarly to our previous studies in *Arabidopsis*, our primary interest in the *C. elegans* model was to characterize the previously unknown *C. elegans* innate immune response. In important work, postdoctoral fellows Dennis Kim and Rhonda Feinbaum demonstrated that *C. elegans* has a primitive innate immune system that shares key elements with the innate immune system in mammals.

What is your mentoring style?

I like to give my students and postdocs as much freedom as possible to develop their own projects and to take their projects with them when they leave the laboratory. In general, I do not believe in micromanagement. Thus, I think my laboratory is best suited for students and postdocs who are self-motivated and independent. I have had the good fortune of having many outstanding students and postdocs in the lab who have gone on to have very successful careers of their own. As I near the end of my career, I feel much more proud about my many successful mentees than any particular scientific contribution from my laboratory.

What do you enjoy most about being a scientist?

I am more interested in the process of doing science than in any particular result. Similarly, I am much more interested in the big picture than the mechanistic details of any particular biological process. This is reflected in my style of doing science and explains why I keep on developing new systems to work on in the lab. When a particular project in the lab gets to the point where referees start wanting to know mechanistic details, I know that it is time to start working on something new.