Profile of Jeffery L. Dangl

If you make the mistake, as some do, of calling Jeff Dangl a botanist, his embarrassment is palpable. Trained as a mouse immunologist, he admits to a "certain lack" of botanical knowledge. The John N. Couch distinguished professor of biology and adjunct professor of microbiology and immunology at the University of North Carolina, Chapel Hill, will, however, give you an earful about the lack of respect, and with it, funding, historically given to the plant sciences compared with his former field of immunology.

He studies *Arabidopsis thaliana*, a fast-growing, weedy plant known as thale cress, which has become the *Drosophila* of plant-based molecular biology. Long before it became the first plant to have its entire genome sequenced, Dangl was among a handful of researchers who pioneered its use as a model system for studying plant disease resistance, not solely as botanists but using all of the powerful tools of molecular biology, genetics, and plant pathology combined. He was at the cutting edge of what he calls a mini-revolution that not only showed that plants have an immune system, but that, at the genetic and molecular levels, the system shares basic organizational traits with mammals.

For his work in deciphering how plants interact, at the molecular level, with pathogens to fight off disease, Dangl was elected into the National Academy of Sciences in 2007. In his Inaugural Article (1), Dangl, his graduate/postdoctoral student David Hubert, and colleagues build on that work using genetics techniques to show how three chaperone molecules interact to control the levels of nucleotide-binding domain and leucine-rich repeat (NB-LRR) proteins—critical intracellular receptors for proper immune function in plants.

From the Hospital to the Lab

Dangl grew up in Redding, northern California, surrounded by mountains, lakes, and streams and close enough to the ocean for frequent visits. His mother, a teacher and book-store clerk, and his father, a high school teacher and administrator, often took him and his younger brother and sister hunting, fishing, and camping in the surrounding area.

Dangl vividly remembers one family outing when he got his first glimpse of "the coolness of science." His father caught a salmon and cut it open so he could see what it had eaten. "The fish's heart was still beating," recalls Dangl. "I was in second grade and I thought that was pretty cool. I got really interested in science after that."

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His curiosity took a serious turn in his early teens when he found out he had inherited a rare form of muscular dystrophy: facioscapulohumeral muscular dystrophy (FSH). During his teen years, Dangl spent time in research hospitals, once at the University of California, Los Angeles, and once at the University of California, San Francisco.

"I went at the hospitals' invitation," he explains. "I wasn't sick and had a lot of time on my hands, so I visited a lot of labs. The researchers were incredibly accommodating and open. I looked at my own muscle cells under the microscope and began to get an interest in pathology and real lab science."

At Rancho Los Amigos, a leading pediatric orthopedics hospital in Downey, CA, the only bed available for Dangl was in the burn and back surgery recovery ward. "I was sharing a ward with kids who had been through really nasty stuff," he says. "I ended up helping out a little and realized quickly that I wasn't cut out for helping people in clinical settings. I got too emotionally involved."

Along with his experience in research hospitals, Dangl was influenced by his high school chemistry teacher, Jon Lefler. "We did lots of experiments; I got to blow things up," says Dangl. "He was a lot of fun."

Lefler encouraged Dangl to apply to Stanford University. Although, even with scholarships and work study, Dangl went into debt like most of his friends, he says, "I would like to say that to folks these days who worry about university-related debt, forget it—it's the best investment you'll ever make."

A Serendipitous Summer

Stanford was daunting, remembers Dangl. "It's hard to be the smart kid at a small school. But to be a smart kid among hundreds of other smart kids is something altogether different," he says. "At Stanford, you get the ego beat out of you in the first five days. It's a bit of an existential moment. But then you realize that you are going to survive, and it's extremely liberating."

It is also a good lesson for someone hoping to make it in the world of research. "You lose a lot more than you win in research," says Dangl. "Experiments don't work often, projects crash and burn, papers get rejected, and there is usually someone out there smarter and working harder than you."

Science was not Dangl's only interest. He double-majored in biology and modern literature.

At the end of his sophomore year, he received his big scientific break. He did not want to go home for the summer but needed a job to pay the rent. He met Ron Levy in Stanford's medical school. Although Levy had no money to hire Dangl, he suggested he talk to Len Herzenberg, cocreator of the FACS. Herzenberg's lab was using the FACS to sort suspended cells by size and type. He hired Dangl to run the sorter weekends and nights.

"It was awesome," says Dangl. "I never looked back. I learned to run the FACS. I also got to work with postdoc Sam Black, who taught me a lot of basic lab science, like mouse dissection and antibody purification."

Dangl did his senior research thesis with Herzenberg and after finishing his biology degree, worked in the lab while he took an extra year to complete his English degree. He was applying to graduate schools around the country when he met Sarah Grant, a graduate student in the genetics lab of Stanford's Stan Cohen.

"By the time I was being admitted to graduate school, I had decided I should stay at Stanford," says Dangl, explaining that Grant later became his wife.

He remained in Herzenberg's lab. "It was a very heady time at Stanford," he says. "Recombinant DNA techniques were very new; the first explosion monoclonal antibodies were being produced."

Dangl's PhD focused on immunology. In particular, he and his colleagues were interested in mouse Ig heavy-chain genes, whose protein products determine specific antibody functions. "The problem was, the heavy-chain functions were difficult to study, because they were typically recombined with different

This is a profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 9556 in issue 24 of volume 106.

antigen combining sites," says Dangl. To overcome this difficulty, he used a combination of FACS and recombinant DNA to create a series of monoclonal antibodies where every antigen-combining site was identical and bound to a fluorescent sensor molecule. He wanted that combining site to be hooked up to all of the mouse and human Ig heavy-chain genes so that the heavy chain was the only variable.

"Once we had these molecules, we could study heavy-chain functions like complement fixation and in collaboration with Lubert Stryer, the ability of the different heavy chains to wave the arms of the molecule about the hinge domain," says Dangl (2–4).

"The guy who got me through my PhD was Vernon Oi," Dangl says. "He was a superb scientist and mentor for me. He was the guy who daily pushed me to ask questions and molded me from someone who was interested and excited about science to someone who could think like a scientist. That's the critical transition."

Pioneering Plant Science

By the time Dangl finished his doctorate, the field of molecular immunology was getting crowded and competitive. Dangl had the notion that he wanted to spend time at what was then the world's most vibrant plant molecular-biology institute: the Max Planck Institute for Plant Breeding Research at the Max Planck Gesellschaft in Cologne, Germany, then run by Jeff Schell.

A fateful trip to the library sent Dangl down a path that he is still on. He was looking for a certain paper in PNAS, and the journal opened to a different paper by Klaus Hahlbrock that showed that plants respond to a fungal infection by transcriptionally activating genes required to fight the infection.

"I did a bunch of reading and realized that the molecular mechanism involved in this kind of an immune response in plants was a completely black box," says Dangl. "There was essentially no molecular biology done on these systems at that time but superb genetics from plant breeders that framed the important questions clearly."

Hahlbrock was just moving to a lab at Max Planck in Cologne and offered Dangl a spot. The National Science Foundation (NSF) provided funding in the form of a postdoctoral fellowship meant to bring molecular biologists from outside plant biology into the field. "This NSF postdoc program was incredibly effective," says Dangl, noting that many other former postdocs are now National Academy of Science (NAS) members or science-policy leaders.

Once there, Dangl created an easy-to-use system to study how UV light and pathogen stress triggers transcriptional changes, leading to plant defense genes (5). Schell was just beginning to use *Arabidopsis* for a wide range of studies, and Dangl saw the plant's potential as a model for plant immunology.

He pursued that idea during a fully funded 6-year position at the Max Delbrück Institute, which opened in Cologne as Dangl's fellowship ended. "I was a happy guy," he says, explaining that the job was designed to jumpstart the careers of junior researchers. "I had assistant professor status with full funding for six people and no teaching responsibility." His wife got a similar job, and they stayed in Germany a little more than 9 years.

Even talking about a plant-based immune system was the beginning of a minirevolution.

"We loved it," says Dangl. "We were there when the [Berlin] wall came down. The 18 months around that were amazing. We also traveled and met a lot of great scientists who became close friends."

Dangl used the time, relatively free of funding worries, to work hard and make his mark. He showed that he could use molecular biology to study how *Arabidopsis* fights off pathogens and that, in turn, could help develop a model of plant immunology.

Even talking about a plant-based immune system was the beginning of a minirevolution. Until the mid-1980s, most people thought that only highly evolved vertebrates had immune systems. Then, researchers began to discover that even some of the oldest organisms on Earth had ways to recognize and combat pathogens.

Of course, says Dangl, breeders had long recognized that some plants could resist diseases that others could not and that resistance could be bred into nonresistant plants. However, the molecular biology of those systems was completely unknown.

Dangl started looking for the molecular mechanisms that plants use to respond to pathogens, many of which inject proteins called effectors into plant cells, wreaking havoc and allowing the pathogen to infect more cells. Unlike humans, plants do not have immune cells circulating around looking for infections. Rather, each cell needs to detect pathogens and alert neighboring cells. They respond by reprogramming their transcriptional output, synthesizing a suite of toxic compounds to deter further invasion, and sacrificing some cells at the infection sites. This suite of responses stops pathogen growth.

Over the years, people described mutants in maize and other crops that had an uncontrolled hypersensitive cell-death response. For Dangl, this finding indicated that there must be some type of genetic control that negatively regulated cell death.

He and his colleagues successfully identified *Arabidopsis* mutants that could not control cell death (6) as well as loci that could control pathogen recognition (7). They also cloned one of the first disease-resistance genes (8).

"That was good," he says. "It allowed us to start to build a model of what a plant immune system looked like."

Guard Hypothesis

The work gained Dangl notoriety among his peers as his 6 years wound down in Cologne, and he and his wife started applying for jobs back in the United States. Dangl dreamed about going back to California. However, plant biologist Ralph Quatrano, then the head of biology at the University of North Carolina (UNC), Chapel Hill, suggested he think about UNC. Dangl and his wife both got jobs at UNC and have shared lab space and more recently, research interests.

"It's a wonderful place," he says. "It's a real biology department that additionally has a great relationship with the medical school. It's a very good fit."

Soon after he arrived in 1995, his lab isolated several plant resistance proteins that respond to infection by initiating plant cell death (8). The proteins turned out to be NB-LRR proteins, which are a class of proteins now known to mediate pathogen recognition, activate defense responses in plants, and have analogs in animals.

Oddly, there appeared to be far fewer disease-resistance proteins in *Arabidopsis* than there are likely pathogen virulence factors, making theories that each pathogen effector protein met up with a corresponding disease-resistance protein difficult to reconcile. "We had a repertoire problem," Dangl notes.

Dangl surmised that plant disease-resistance proteins must, therefore, respond to something broader than pathogen effectors. He, together with his colleague Jonathan Jones at the Sainsbury Lab in Norwich, United Kingdom, proposed that they instead monitor the integrity of molecular machines in the plant cells, looking for damage or modifications to proteins within the cell—modified self, a term Dangl borrowed from mammalian immunology. Based on this concept, Dangl and Jones developed the guard hypothesis (9, 10), suggesting that each disease-resistance protein

guards a specific host protein and reacts when pathogens modify them.

In a series of experiments, Dangl and his colleagues presented experimental evidence supporting the guard hypothesis. They proved the theory of modified self by showing that certain plant NB-LRR resistance proteins kicked in when pathogen effector proteins damaged proteins with which they were associated in the cell. For example, when the effector protein AvrRpt2 cleaves a small *Arabidopsis* protein called RIN4, the associated RPS2 NB-LRR receptor is activated (11). Dangl and his colleagues showed that RIN4 is genetically a negative regulator of NB-LRR activation, and when it is modified (12), these modifications lead to the activation of different NB-LRR receptors.

Through the years, Dangl, among many others, has also shown that the NB-LRR proteins are evolutionarily conserved across all plants and extend to proteins that mediate various processes in mammalian immunity.

It turns out that there are three chaperone molecules that control the steady-state level of NB-LRR proteins. These chaperones, known as HSP90, SGT1, and RAR1, interact with each other and are necessary for NB-LRR protein regulation. In Dangl's Inaugural Article (1), he and his team teased apart some of the mechanisms by which the chaperones regulate NB-LRR. To start, David Hubert conducted a "deep genetic screen of *Arabidopsis* in more than a million seedlings, looking for rare phenotypes," says Dangl.

"That allowed us to zoom in on how the two of the three chaperone molecules work to control NB-LRR protein levels," he says. "It's an appealing paper that answers with genetics a mechanistic question."

Indeed, they found two mutations of the HSP90 protein that completely suppressed *rar1* mutation. By examining what happens in the HSP90 mutants, they deduced that RAR1 physically enhances the transition state of HSP90 as it moves from a lid-open conformation to a lid-closed conformation.

"This finding extended our earlier finding that RAR1 and SGT1b cochaperones antagonize each other's function to control NB-LRR protein accumulation (13) and placed the likely site of that antagonism at the HSP90 lid domain," says Dangl.

Convergence and Applications

As Dangl's lab digs deeper into the specific mechanisms of the plant immune system, they have also played a large role in showing the convergence between immunity in animals and plants.

"In the last 10 years, a nice thing that's been intellectually rewarding is that animal innate and plant innate immunologists can learn from each other," says Dangl.

In fact, findings from plant biology, including work from Dangl's lab, led researchers in France to discover a mutated form of a disease resistance-like protein that is partially to blame for Crohn's disease in humans. Additionally, Dangl finds that more and more people have begun to talk about the connections that can be made between plant and animal work. On the plant front, Dangl believes that the field is entering "the years of application."

"We think we know enough now to begin doing things predicatively," he says. "Researchers can take, for example, the potato-blight pathogen and find all of its virulence factors and use those to screen wild potatoes, which are resistant to the blight, for genes that will allow us to breed good resistance into commercial potatoes."

With increasing pressures on water and food supplies, Dangl believes this kind of work will be critical. He is on the scientific advisory board of 2 Blades Foundation, which supports the development and agricultural use of disease-resistant crop plants.

Although he looks forward to seeing his lifetime of research applied in ways that can benefit the world, he is most proud of the people who have gone through his lab and the impact that these scientists are having on the fields of plant biology, molecular biology, and immunology.

Beth Azar, *Science Writer*

1 Hubert DA, He Y, McNulty BC, Tornero P, Dangl JL (2009) Specific Arabidopsis HSP90.2 alleles recapitulate RAR1 cochaperone function in plant NB-LRR disease resistance protein regulation. *Proc Natl Acad Sci USA* 106:9556–9563.

2 Dangl JL, Parks DR, Oi VT, Herzenberg LA (1982) Rapid isolation of cloned isotype switch variants using fluorescence activated cell sorting. *Cytometry* 2:395–401.

3 Oi VT, et al. (1984) Correlation between segmental flexibility and effector function of antibodies. *Nature* 307:136–140.

4 Dangl JL, et al. (1988) Segmental flexibility and complement fixation of genetically engineered chimeric human, rabbit and mouse antibodies. *EMBO J* 7:1989–1994.

5 Dangl JL, Hauffe KD, Lipphardt S, Hahlbrock K, Scheel D (1987) Parsley protoplasts retain differential responsiveness to u.v. light and fungal elicitor. *EMBO J* 6:2551–2556.

6 Dietrich RA, et al. (1994) Arabidopsis mutants simulating disease resistance response. *Cell* 77:565–577.

7 Debener T, Lehnackers H, Arnold M, Dangl JL (1991) Identification and molecular mapping of a single *Arabidopsis* locus conferring resistance against a phytopathogenic *Pseudomonas* isolate. *Plant J* 1:289–302.

8 Grant MR, et al. (1995) Structure of the Arabidopsis RPM1 gene enabling dual specificity disease resistance. *Science* 269:843–846.

9 Dangl JL, Jones JDG (2001) Plant pathogens and integrated defense responses to infection. *Nature* 411:826–833.

10 Jones JDG, Dangl JL (2006) The plant immune system. *Nature* 444:323–329.

11 Mackey D, Belkhadir Y, Alonso JM, Ecker JR, Dangl JL (2003) Arabidopsis RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. *Cell* 112:379–389.

12 Mackey D, Holt BF, 3rd, Wiig A, Dangl JL (2002) RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. *Cell* 108:743–754.

13 Holt BF 3rd, Belkhadir Y, Dangl JL (2005) Antagonistic control of disease resistance protein stability in the plant immune system. *Science* 309: 929–932.