Profile of Joseph R. Ecker

In a Greek translation of the Old Testament, a prophet named Amos said, "I was a herdsman and a piercer of figs," a reference to the ancient practice of slashing a fig to hasten ripening. The cut fig rots, but as it does it emits a gas that ripens other unmarred fruit. "Amos was out in the field inducing an ethylene response," speculates Joseph R. Ecker, a professor at the Plant Biology Laboratory at The Salk Institute in La Jolla, CA, who has pioneered the understanding of ethylene's role in fruit ripening, pathogen defense, and germination in plants. "So that's the first commercial application you can find of ethylene technology," Ecker says.

Ecker, elected to the National Academy of Sciences in 2006 and a member of the PNAS Editorial Board, has spent much of the last 25 years dissecting the genetics and identifying key signaling components of the ethylene pathway in plants. He was an early advocate of and participant in sequencing the genome of *Arabidopsis thaliana*, which gained a foothold as the plant world's model organism only in the late 1980s. He has also developed many genomic tools and resources for *Arabidopsis* researchers, such as chips to identify all the transcripts in the genome and a collection of *Arabidopsis* plants carrying mutations for almost every gene, which have revolutionized plant biology. In his Inaugural Article in a recent issue of PNAS (1), Ecker and his colleagues describe the role of a member of the ethylene pathway.

Attic to Laboratory

Ecker's interest in all things science was nurtured by a series of influential coincidences. The first was the fact that his birthplace in Mount Carmel, PA, was in the middle of coal mining country. The region was punctuated with massive boulders that Ecker would explore and chip at with stones and knives to reveal fossilized leaves. "It was fascinating that you could find something millions of years old, and you were the first person to see it in its fossilized form. It was perhaps my first feeling of a sense of discovery," he says.

When Ecker was 6 years old, his family moved to a house in Trenton, NJ that had previously belonged to an eccentric engineer. The house was filled with a tantalizing assortment of chemicals and scientific tools, such as jars of mercury, sophisticated blown glass tubing, and electronic equipment. Ecker

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and his older brother David moved the equipment into the attic, which they transformed into a make-shift chemistry laboratory for science projects.

When it came time for college, Ecker anticipated becoming an engineer, but "I didn't quite fill out my application forms," he says. His brother David completed his applications for him and selected biology as his major. "I got back this acceptance that said 'biology.' I said, 'What?'" His brother encouraged him to give biology a try.

Ecker attended the College of New Jersey (Ewing, NJ), where biology proved to be a good match, and earned a bachelor's degree in biology in 1978. One of the most valuable aspects of his undergraduate education was the opportunity to dabble in different fields. "Every class you took had a laboratory, from mammalian comparative anatomy to physiology to microbiology to genetics," he says. "There was a real chance to do hands-on science, . . . [such as] electron microscopy on rat hearts. . . I worked on *Drosophila*, pushing flies, doing crosses. . . measuring oxygen levels from cells in physiology. . . extracting plasmids and looking at them under an electron microscope."

Ironically, Ecker did not take any plant biology courses during his collegiate years. The reason was that these courses were among the only ones without an experimental laboratory component, he says. "I used to say that the only things that plants are good for is looking at and eating, then I turn around and that's what I ended up spending my life doing," he says.

Taste for Genomes

Biology instilled a deep interest in genomes in Ecker, leading him to enter a Ph.D. program in microbiology at the Pennsylvania State University College of Medicine (Hershey, PA). He joined the laboratory of virologist Richard Hyman and studied herpes viruses, anticipating that the viruses would be a good model for understanding regulatory processes and genomes of other organisms. His doctoral research focused on the structure of varicella zoster, the virus that causes chicken pox. "As children, everyone got chicken pox, but we didn't know anything about its genome. That fascinated me," he says. Ecker's research led him to discover that two major forms of varicella zoster existed (2).

As Ecker searched for a postdoctoral fellowship after finishing his Ph.D. in 1982, he unknowingly began his transition into the plant world. While looking for a stimulating paper to present to his colleagues for the weekly journal club, he came across two papers by Mary-Dell Chilton and others (3, 4) that described an interaction between the microbe *Agrobacterium* and a plant cell that eventually led to a tumorous condition called crown gall. "This was fascinating, really unexplored territory. . . something like SV40 integrating into the genomes of man," Ecker says. He applied for a postdoctoral position in Chilton's laboratory, but no openings were available.

Ecker's advisor told him that Ron Davis at Stanford University (Stanford, CA), a well known yeast geneticist and graduate-school colleague of Hyman's, was planning to start work on plants, in particular *Arabidopsis*. Ecker contacted Davis, who told him that he was interested in working on plant hormones and sent Ecker a two-page review on ethylene gas. Ecker was determined to study something unique to plants, and given that humans do not produce ethylene gas, this area appeared to be a good place to start. At the same time, the National Science Foundation (NSF) was encouraging new doctoral recipients to enter plant biology by offering 3-year fellowship grants. Having never taken a plant course, Ecker drove to Penn State's main campus and immersed himself in plant journals for several days before penning a proposal to purify the enzymes involved in ethylene synthesis.

Ecker received the NSF fellowship and drove cross-country to begin working with Davis at Stanford. At the time, modern plant biology was in its infancy, with the most striking aspect being the lack of a model organism. Davis's laboratory resembled a kitchen with food processors adorning the bench tops, as

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This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 13286 in issue 36 of volume 103.

people studied peas, corn, squash, and carrots. "We really started from ground zero for most plants. There were no cDNA libraries, no lambda or plasmid genomic libraries," Ecker explains. But Davis was a proponent of technology development, and many students and postdoctoral fellows in his laboratory created useful resources like cloning vectors and yeast artificial chromosome (YAC) libraries.

After a brief period studying maize, Ecker shifted to a cell culture system based on carrot roots. Carrot cells were favorable for studying ethylene response, because only 5–10 minutes of exposure can trigger the rapid activation of gene expression. Much of what Ecker knows about the physiology of ethylene was learned from another postdoctoral fellow in the laboratory, Athanasios Theologis. "He's been a friend and collaborator ever since and a coauthor on many papers," Ecker says.

Ecker devoted considerable time to studying gene expression in the carrot cell cultures and developing electroporation procedures for plants. "[But] we didn't have a good genetic system, which led us to wonder, 'How do we silence genes that we have cloned?'" he says. This question led Ecker and his colleagues to prove for the first time that antisense technology could be used to regulate gene expression in plants (5).

Carrots eventually proved to be an inconvenient model for genetics, because crosses required placing the flowering plants into large bags of netting and releasing large meat flies to carry out the pollination. By the mid-1980s, a critical mass of senior biologists had adopted *Arabidopsis* as the model plant, in large part because it could be easily transformed and had a relatively small genome. These advantages precipitated the development of genomic resources, including an *Arabidopsis* YAC library that Ecker created.

Down the Ethylene Pathway

Ecker completed his fellowship and secured his first position in 1987 as an assistant professor at the Plants Science Institute at the University of Pennsylvania (Philadelphia, PA). He left Stanford with a bounty of mutagenized *Arabidopsis* seeds, and at the University of Pennsylvania his first experiment initiated screens for mutations that altered the ethylene response. "With ethylene you get an immediate response, and within hours you can see changes in growth," he says. "You plate the seeds, put them in the dark with ethylene, [and] 3 days later you can see mutants."

These mutants became the bedrock of Ecker's career. Shortly after arriving at the University of Pennsylvania, Ecker grew approximately 10,000 mutagenized *Arabidopsis* seedlings in ethylene on a Petri dish. Most of the seedlings were only approximately 1 mm high, but some mutants towered above at 5–10 mm, resembling tiny trees dotting a tiny grassy field. "I showed this to Kelly Tatchell, he's a true geneticist, and he said, 'That is your future. . . you stick with that,'" Ecker says. Almost 20 years later, this growth technique is still one of the primary screens used to identify mutants (6).

Since that time, Ecker has focused primarily on two research pursuits: genetically and biochemically dissecting the ethylene pathway, and developing genomic technology for studying *Arabidopsis*. A particularly challenging aspect of ethylene research is that its targets and function vary with developmental stage. Ethylene changes plant morphology during germination, triggers a defense response against pathogens, and controls fruit ripening and causes petals to fall off. "We are really trying to understand what are the different signals that contribute to these different outcomes," Ecker says.

"[Prophet] Amos was out in the field inducing an ethylene response."

One of the first signaling genes to emerge from the mutagenesis screens was CTR1, a negative regulator of the ethylene response pathway and a member of the Raf family of protein kinases. "As soon as we saw this, we thought this pathway is going to be the same as the one in mammals. . . this was the first clue that plants are going to have both common and unique features," Ecker says (7).

He used the physiology and genetics of plants to dissect the ethylene pathway and anticipate the position and roles of various players, culminating in an article published in 1995 (8). Although the molecular identity of many of the players would not be revealed for years to come, Ecker says that all the predictions made in that paper have proved correct. "It's one of the papers I'm most proud of," he says.

Sequencing *Arabidopsis*

As Ecker probed the ethylene pathway, he was equally committed to expanding the technological toolbox for ways to dissect *Arabidopsis*. "If there was a theme or message in my postdoc with Ron Davis's lab, it was that there was a biology–technology cycle. That meant that you are going to hit a roadblock to studying the interesting biology, especially with a new organism, if you don't develop the tools," Ecker says.

Since beginning his first professorship, Ecker has expended at least half of his efforts in developing genomic technology. His laboratory created the third YAC library, this one specifically for *Arabidopsis* (10), and he began identifying microsatellite markers to anchor physical maps of the plant's genome (11). The approach was mandated by necessity, Ecker says, because he could not localize his ethylene mutants without maps. Following the YAC libraries were bacterial artificial chromosome (BAC) libraries, which then led to a finer resolution map.

But for Ecker, these crude maps were just the beginning. "It was clear that methods were going to evolve, and what was needed was to rally people around sequencing," he says. He made his opinion known at the Fourth International Conference on *Arabidopsis* Research in Vienna, Austria, in June 1990. Although he was a new assistant professor and "had a hard time getting the ear of more senior colleagues," he says, his dedication to creating research tools, specifically YAC and BAC libraries and maps, gave him credibility and propelled him to the forefront of the *Arabidopsis* Genome Initiative.

During the Vienna meeting, the researchers forged a 10-year plan for sequencing *Arabidopsis*, which was later submitted to NSF. This plan later became the *Arabidopsis* Genome Initiative, which launched in 1996. During 1998–1999, Ecker held the reins as chairman of the *Arabidopsis* Genome Sequencing Committee. Initially, he says, he was daunted by the prospect of coordinating so many people and approaches in different locations. "In the end, people had a common vision," he says, which fostered a refreshing and unexpected degree of communication and collaboration.

As the genome effort drew to a close, The Salk Institute lured Ecker away from the University of Pennsylvania. "Penn had been good to me but was moving a bit too slow on the genomics front. . . whereas Salk/UCSD/Scripps and the local biotechs make for a very strong plant biology group," he says. As

it became clear that the genome would be completed in 2000, 3 years ahead of schedule (12, 13), Ecker began probing the yeast community to grasp how the yeast genome sequence had impacted their research. "I didn't see any huge development. I was actually thinking we were going to be disappointed," he says, "but behind the scenes the yeast guys were developing some of the most powerful tools that we are still hoping to develop for *Arabidopsis*." In 1998, before the genome was finished, Ecker and his colleagues cobbled together a plan, the 2010 project (14), describing how to use the plant's genome sequence. "I was convinced we had to study the genome in a way never done before, so no more 'one by one;' we needed a new system to look at all the genes in a single assay," he says.

That realization led Ecker to team up with chipmaker Affymetrix (Santa Clara, CA) to start building a single chip with the entire *Arabidopsis* genome. The arrays were composed of consecutive 25-bp segments that essentially tiled the genome. "One thing that became very clear when we were analyzing the sequence is that we couldn't find the genes," Ecker says. From computational analyses of the genome and comparisons with known genes, Ecker and his colleagues found that many annotations were not accurate, such as incorrect predictions of splice sites, missed exons on the 5′ or 3′ end, or some genes missed completely.

Since 2000, Ecker and colleagues have developed new gene scouting techniques. "Because if you sequence the genome with the goal of finding all the genes, you've got to find them," he says. One approach has been to probe the tiling arrays with labeled RNA transcripts. "The surprise is that we found ones we didn't expect: lots of genes in the intergenic space that are not coding for proteins, lots of genes coming from the antisense strand that we really don't know anything about," he says. Ecker says the paper that resulted from the analysis is his "coolest" to date (15). Tiling arrays are now used for all organisms because they provide an unbiased screening tool and more accurate view of genomes.

Library of Mutants

"One of the crucial genetic tools for any organism was targeted gene replacement. . . , and many postdoc and grad student lives have been lost to trying to get targeted gene replacement working at a high enough efficiency in *Arabidopsis*," jokes Ecker. He and his colleagues decided to use the *Agrobacterium* system that he had been using to screen for mutants in the early 1990s. *Agrobacterium* T-DNA inserts itself in the genome randomly into almost every known gene as well as many intergenic regions. "If we had picked the 25,000 [sites] we thought were genes, we would have missed all these other things," he says, "so with a random approach, we have lots of insertions in intergenic regions full of microRNAs and different transcripts of unknown function."

Ecker and his team individually mapped and indexed each of the 150,000 *Arabidopsis* mutants carrying a T-DNA insertion. The contribution of mutants from other laboratories now brings the total to ≈400,000 mutants, and researchers worldwide can go online and order a mutant for any gene. The effort has created a critical resource for *Arabidopsis* researchers in academia and industry, and Ecker believes it is probably the aspect of his work that has had the greatest impact to date (16).

All of these tools have proved useful for studying the ethylene pathway. In his PNAS Inaugural Article (1), Ecker used the tiling arrays to identify the targets of the ethylene-insensitive mutant EIN5, a 5′-to-3′ exoribonuclease. The tiling arrays revealed that in EIN5 mutants a build-up of EBF1 and EBF2 mRNAs occurs, which ultimately suppresses the action of EIN3 (17, 18) and blocks the ethylene response producing tall mutants. Although Ecker is not personally involved in commercial applications of the ethylene pathway, he says that an increasingly fine-scale understanding will allow engineering of targeted mutations that manipulate specific arms of the ethylene pathway, such as those involved in fruit ripening, without disrupting other essential functions of the hormone.

As for his future, the top of Ecker's "to-do" list involves characterizing the *Arabidopsis*"methylome." As in humans, the methylation status of a gene in *Arabidopsis* can have a profound impact on the plant's phenotype. Ecker's laboratory is thus expanding into studies of epigenetics. He also hopes to exploit ultra-high-throughput sequencing tools to fully sequence more ecotypes of *Arabidopsis* and then correlate unique genomes sequences with the unique properties of each particular plant.

With all his achievements, however, Ecker confesses that his green thumb does not extend beyond the Petri dish. "In fact, I'm trying to grow lemon trees in San Diego and having a tough time. I've got two in the backyard, and they are just the sickest looking things."

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