

Steve Howell

How did you spend your career?

I spent my career largely in academia. My first position was in the Biology Department at the University of California (UC) San Diego, my next position was with the Boyce Thompson Institute at Cornell, and my final position was in the Plant Sciences Institute at Iowa State University. I had wonderful sabbatical experiences at the John Innes Institute in Norwich, United Kingdom, and CSIRO in Canberra, Australia. I also had a stint as division director with NSF in Washington, DC. For the past 11 years, I have headed up the scientific advisory board of Cibus, a plant gene editing company in San Diego. So over the years, I have seen, participated in, and led plant science from many different perspectives.

What do you consider to be your most important contributions to plant science?

My most satisfying contribution was writing the book *Molecular Genetics of Plant Development* (Howell, 1998), which was used as a text by many budding plant biologists growing up during the late 1990s to 2010 or later. My most publicized contribution was work done by David Ow in my lab putting the firefly luciferase gene into plants. Images of the glowing tobacco plant made the *New York Times*, *Time* magazine, the Today Show, *Science* magazine, a CD cover, T-shirts, and my daughter's high school science textbook. My most important scientific contribu-



tion was being the first to introduce biologically active recombinant DNA, cauliflower mosaic virus (CaMV) DNA, into plants.



Glowing transgenic tobacco plant expressing a construct in which the CaMV 35S promoter drove the firefly luciferase gene. The firefly gene was cloned by Marlene DeLuca and Don Helinski and used by David Ow to generate a transgenic plant. The photo was produced by Keith Wood, who soaked the plant in luciferin, the luciferase substrate, and laid it out overnight on a photographic plate to produce this iconic image.

The introduction of CaMV DNA into plants is an interesting story, steeped in controversy. The controversy surrounded the biological activity of cloned plant viral DNA in plants.

CaMV has a small circular 8 kb DNA genome composed of six or seven genes (depending on how you count them). The CaMV

genome (called CLMV at the time) was first cloned in the lab of Charles A. Thomas Jr. at Harvard. They reported in *Science* (Szeto et al., 1977) that the cloned viral DNA appeared to be a true copy of the original DNA obtained from the virus; it had a similar restriction enzyme pattern compared with virion DNA. However, they found that the recombinant viral DNA was not infectious when they tried to introduce it back into plants. They reported that this was the case whether they had excised the viral DNA out of the plasmid vector or not.

The paper from Thomas's group was unusual. The authors stated in the report, "We attempted to see whether the recombinant plasmid was replicated in plants. The results

indicated that the first experiment was successful (although the cloned DNA could not be demonstrated to be infective in plants). The second experiment gave inconclusive results. . . . Since this experiment has been halted for non-scientific reasons, we report

on progress to date." The nonscientific reasons for halting the research apparently included the fact that NIH withheld Thomas's funds for recombinant DNA research because he failed to obtain NIH certification for his laboratory. According to a report in the *Harvard Crimson* (June 26, 1978), "Thomas's \$112,000 grant

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was proscribed when NIH found he had failed to file a Memorandum of Understanding and Agreement (MUA) with the Institutes.”

Nonetheless, in the Science paper, Thomas’s group put forward three possible reasons why the viral DNA was not infectious (Szeto et al., 1977). One reason had far-reaching implications—that the viral DNA might have some crucial secondary structure that was not present in the cloned copy of the viral DNA. The latter sent some shock waves through the plant molecular biology community. Was there something different about CaMV DNA when it was cloned? Because the viral DNA was that of a plant virus, did that also mean that there is something different about plant DNA when it is cloned? Would one never be able to clone plant DNA in biologically active form? What is it about plant DNA that might be altered when it is propagated in a bacterial plasmid?

Undeterred and perhaps youthfully naive, I set about to find out for myself whether cloned CaMV could infect plants. But given the misfires described above, my chances for infecting plants with cloned CaMV DNA seemed pretty bleak. So I was mightily surprised when, on a June afternoon soon after my family and I had returned from a two-week vacation, I found that the plants I had inoculated before vacation showed symptoms of viral infection. Before vacation I had inoculated some turnip plants with recombinant CaMV DNA excised from a plasmid by cutting at the single Sall restriction enzyme site that had been used

to insert the viral genome into the plasmid. Plants were inoculated by rubbing leaves with the cloned CaMV DNA in a suspension of an abrasive called Celite. This is the typical way virologists propagate viruses that can be mechanically transmitted, essentially by inoculating plants with virus introduced through scratches made by the abrasive on the leaf surface.

I had inoculated several plants with the cloned CaMV DNA, including a mock-inoculated control. So when I walked into the greenhouse, the first thing I noticed was that some of the plants were infected and had viral symptoms. CaMV symptoms in turnips are quite obvious, with prominent vein clearing and infected leaves that have “green islands” and yellowed veins. My first thought was, “Oh, rats; the plants have been contaminated.” However, when I looked more carefully, it was only the plants inoculated with recombinant CaMV DNA that showed symptoms. That made my heart skip a beat, but I was still wary. In the field, CaMV is normally transmitted by insects—aphids. What if aphids had transmitted the virus in the greenhouse and infected only plants that had been inoculated with the cloned CaMV? The solution to this problem was to repeat the experiment with an aphid nontransmissible strain (CM4-184)—and the next time gave the same results.

It was quite unexpected to see that the recombinant CaMV DNA had infected plants. The CaMV genome is circular, and the circle had been opened to clone the

DNA into a bacterial plasmid. The full-length cloned genome was excised as a linear piece of DNA by cutting the plasmid at the Sall sites, the same sites that had been used to insert the viral DNA into the bacterial plasmid. A characteristic of cutting DNA with Sall is that it leaves “sticky ends.” The sticky ends are 4 base DNA overhangs that can be joined together by DNA ligase. However, the recombinant CaMV DNA that was used to inoculate the turnip plants had not been ligated to re-form the circular genome; it was introduced as linear DNA, although with sticky ends. Because the full-length viral DNA was infectious and recovered as circular DNA from infected plants, this meant that plants must be able to repair or rejoin the DNA sticky ends. We also tried to inoculate plants with a full-length CaMV genome that had not been excised from the bacterial plasmid. This time we did not see symptoms—meaning that the viral genome could not excise itself when present in full-length form in a recombinant plasmid.

Unfortunately, we never heard why the cloned CaMV DNA from the Thomas lab was noninfectious. The most reasonable explanation was that a small bacterial insertion (IS) element jumped into the cloned CaMV DNA. At the time, bacterial hosts for recombinant DNA work had not been purged of small IS elements, which can inactivate genes not under selection pressure. An IS element might have jumped into and disrupted one of the genes that is needed

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for viral replication, and it might have been small enough to avoid detection in restriction fragment analysis. Fortunately, our cloning was not troubled by IS elements and was carried out by my talented assistant, Linda Walker, and a post-doc, Keith Dudley, who were coauthors on our Science publication (Howell, Walker, and Dudley, 1980).

The availability of an infectious clone gave rise to many other experiments. One of the motivations for working on CaMV was to use it as a vector for introducing foreign DNA into plants. (This was before the successes with *Agrobacterium*.) So we looked for places in the genome where we could insert foreign DNA (small linkers) and found that we could insert linkers into gene VII without losing infectivity. Gronenborn et al. (1981) later found that gene II, encoding the insect transmission locus, could also be interrupted. However, we were unable to insert larger pieces of DNA.

Because of the size limitation, we explored an approach developed Goff and Berg (1976) using an SV40 helper virus system with a vector and a helper virus genome. In the helper system, a disabling mutation in the vector can be complemented by a helper virus genome with a disabling mutation in a different part of the genome. In a mixed infection, the vector and helper support each other in trans. When we tried infecting plants with two CaMV genomes with disabling mutations in different parts of the genome, we found, lo and behold, that it worked! However, a big surprise came when we analyzed the viruses from the mixed infec-



Former students, postdocs, and staff in the Howell lab who were important contributors to the CaMV project. Clockwise from upper left: Linda Walker, Keith Dudley, Joan Odell, Richard Walden.

tion. They no longer carried the mutations; instead, the genomes had recombined with each other and kicked out the mutations. That did not bode well for the development of a virus system to introduce foreign DNA into plants, because recombination between the viral vector and the helper virus would eliminate the foreign DNA carried by the vector.

Although the CaMV recombination problem really befuddled efforts to establish a helper virus system, it did offer opportunities to study somatic recombination in plants. In a study published by Richard Walden and I in 1982, we inoculated plants with different pairs of defective CaMV genomes to determine how recombination

occurred. We challenged plants with pairs of genomes that required one or two crossover events to eliminate the mutations they carried and found that both single and double crossover events occurred in plants.

The recombination proficiency between viral genomes in a mixed infection inspired us to think about how the CaMV genome might be able to excise itself from uncut recombinant plasmids. In earlier studies, we had observed that the full-length CaMV genome could not excise itself

from an uncut plasmid. To do so would require a very precise DNA recombination event at the cloning site. So we cloned partial multimeric genome constructs (Walden and Howell, 1983). (These constructs are greater than full length and created by joining an additional piece of the genome to a full-length genome.) We found, indeed, that turnip plants could be infected with uncut plasmids bearing partial multimeric genomes. From these multimeric constructs, the CaMV genome could excise itself by a single crossover event.

At the time, it was not understood how CaMV genomes replicated and why they were so recombinogenic. Roger Hull and Simon Covey

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(1983) provided a possible explanation. They proposed that CaMV replicated its DNA through an RNA intermediate. They claimed that the large, full genome length RNA (later known as the 35S RNA) transcribed from the viral genome served as a template for the reverse transcription of the CaMV genome. Reverse transcription is known to be a highly recombinogenic form of DNA replication because of the tendency for reverse transcriptase to strand switch when copying RNA to make DNA.

The dreams I had of making CaMV into a useful vector for expressing foreign DNA in plants never materialized. However, we learned a lot of basic information about the introduction of DNA into plants, operation of the CaMV genome, and recombination. Along the way, Joan Odell, a graduate student in the lab, identified a strong promoter in the CaMV genome, the 35S promoter (called the large RNA promoter at the time), which fueled transgenic technology for many years to come.

When did you first become a member of ASPP/ASPB?

I believe I have been a career-long member of ASPB (with a few lapses here and there).

How did the Society impact your career?

I joined ASPP early in my career as a plant molecular biologist. At the time, plant molecular biology was not taken very seriously, and its protagonists were looked down upon by the old-guard plant physiologists. Never was this more apparent than in the establishment

of the Competitive Grants Program at USDA, in which the molecular biologists were considered to be opportunists taking funding from the formula-funded USDA programs. I was one of the early program directors for the Competitive Grants Program, and for our panels we were relegated to a damp basement room with overhead steam pipes in the old USDA building near the Washington Monument.

Later in my career, I was a member of the ASPB Science Policy Committee for quite a number of years. I was kept on not because of any policy expertise on my part, but because I was from Iowa, a state with legislators who had much influence over agricultural funding. In visits with legislators, I found that some of the offices were quite receptive to hearing about ASPB interests and others were not. My interactions with the receptive offices were quite inspiring and made me feel that my efforts to promote ASPB's agendas were worthwhile. These experiences prompted me to become more involved in science funding, and I joined NSF for a couple of years to serve as director for the Division of Molecular and Cellular Biosciences. My major project at NSF was to introduce the area of phenomics to the Biology Directorate and to organize the first workshop on plant phenomics.

What was your motivation for becoming a Founding Member of the Legacy Society?

I want to see ASPB succeed both now and in the future. I am gravely concerned about climate change and the future of our planet. Plants

play a critical role in determining that future, and I want the next generation to understand the importance of plants in shaping our environment and providing us with food, fiber, and feed.

What important advice would you give to individuals at the start of their career in plant science?

Clearly, climate change will motivate much plant science research in the future. Plants can help mitigate the effects of climate change by serving as carbon sinks, and they can also be made to better tolerate the effects of climate change by modulating their stress response systems. When I was at UC San Diego, I lived up the street from Charles David Keeling, whose work in Mauna Loa, Hawaii, on CO₂ concentrations in the atmosphere started the revolution in thinking about climate change. We were working on Rubisco in *Chlamydomonas* at the time, and Dave wanted to know whether we could improve its activity and save the planet. We never made it, but I would advise students to pick up the mantle and start your career to save the planet, even in very little if not big ways.

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