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From Bacteriophage to Plant Genetics

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Abstract

When first asked to write a review of my life as a scientist, I doubted anyone would be interested in reading it. In addition, I did not really want to compose my own memorial. However, after discussing the idea with other scientists who have written autobiographies, I realized that it might be fun to dig into my past and to reflect on what has been important for me, my life, my family, my friends and colleagues, and my career. My life and research has taken me from bacteriophage to *Agrobacterium tumefaciens*–mediated DNA transfer to plants to the plant genome and its environmentally induced changes. I went from being a naïve, young student to a postdoc and married mother of two to the leader of an ever-changing group of fantastic coworkers—a journey made rich by many interesting scientific milestones, fascinating exploration of all corners of the world, and marvelous friendships.

Contents

EARLY EDUCATION

In 1945, I started primary school in a rural area of Austria, where my mother, my sister, and I had moved in order to escape the occupation of Vienna. My father had died during World War II. Several classes of children were combined together, possibly due to a lack of classroom space or teachers. Later primary education and *gymnasium* (high school) were in Vienna. The exciting experiments performed by our chemistry teacher sparked my interest, and I contemplated studying chemistry. Since this subject was somewhat foreign to my artist mother, she tried to find someone who could advise both her and me regarding my interest in pursuing chemistry. I ended up discussing the possibility with a professor in organic chemistry. His main comment was, "Why on Earth should such a young and lovely girl study chemistry (or so)?" So… I studied chemistry at the University of Vienna!

I very much enjoyed studying inorganic and organic chemistry, especially laboratory experiments. A laboratory assistant once asked me to take an impromptu test. At first I refused because I was totally unprepared. He insisted, and in the end I agreed; he handed me a sample of a liquid that I had to identify. I immediately smelled it and realized that it had a smell not as acidic as acetic acid and not as bad as butyric acid—so it had to be something in between: propionic acid! So, without even performing a proper analysis, I passed the test. On another occasion, our endeavors to synthesize butyric acid ended with us students being expelled from the tram. I do not remember how we got home….

I was very grateful to my mother and grandparents, in whose house we lived in Vienna, for supporting me during my studies. In addition, a stipend from Vienna University helped in financially difficult times. Outside activities during these years included going to concerts, dancing, and skiing; we even danced in the long corridors of the laboratory (waltz, of course). While checking my diary from those days, I found an entry dated Saturday, December 12, 1959: "…good conversations with Thomas (Hohn, Chemistry 7th semester)." One of my favorite teachers was Professor Hans Tuppy; he taught biochemistry—then a completely new field of science. His lectures were a delight; so intense, yet so charming! In one such lecture he introduced us to the publication describing the structure of DNA, including its now-famous final sentence, "It has not escaped our notice that the specific pairing we have postulated immediately suggests

a possible copying mechanism for the genetic material" (74, p. 737). We—my colleague Thomas and I—were totally excited and it must have been then that our direction of interest became fixed.

Coincidentally, I just had asked Professor Tuppy to accept me as a PhD student, which he had agreed to do. But by then Thomas had decided to go to the Max-Planck-Institute for Virus Research in Tübingen for his PhD to work with Professor Gerhard Schramm and had asked me to join him. I had a very difficult decision to make. It was even more difficult to crawl to Professor Tuppy to explain to him that I would leave for Tübingen to join my (future) husband. He was extremely generous and wished me good luck for my future.

MAX-PLANCK-INSTITUTE, TÜBINGEN, BACTERIOPHAGE FD

Finding a place to complete my PhD was not easy. Gerhard Schramm did not accept female students. I ended up finding a position in the department of Hans Friedrich-Freksa. He suggested I work on bacteriophage fd but did not suggest any particular scientific field of study for this. This discussion with my professor was one of maybe three during the entirety of my PhD "training." So, I entered the cold water into which I was thrown and looked for supervision. Lack of it turned out to be a problem, at least at first. Once, I started a centrifuge and did not really know how to use it. The rotor broke through the cover and danced through the air—luckily no one was present in the centrifuge room. I was not punished because it was evident that nobody had instructed me. Even so, I am not particularly proud of my experiment.

Bacteriophage fd is a filamentous phage containing circular single-stranded DNA. Since this phage does not lyse its host but only slows down growth of infected bacteria, plaques were difficult to see. The first goal I set myself was to enhance visibility, and I tested various dyes. Giemsa stain won the competition (I kept a bottle of this stain for many years and used it for staining Easter eggs—not such a great idea), and I recovered beautifully blue plaques. This was the basis for establishing the genetic map of part of the phage genome, using plaque types similar to the famous *rII* gene of bacteriophage T4 (2) but much less famous… In any case, this was my first encounter with recombination. This work continued as a study of DNA replication, which caught the attention of Don Marvin, then working in a nearby department, himself an expert in structural biology and still productive (43). We started a collaboration, which was later extended when I moved to the United States.

My time in Tübingen was really exciting. As an example, a collaboration between Alfred Gierer and Gerhard Schramm led to the conclusion that it is the RNA of tobacco mosaic virus and not the protein that causes symptoms in tobacco plants (14). Again and again, I was a lucky witness of scientific breakthroughs in my immediate surroundings. Another source of excitement was the arrival of our son Andreas. I was grateful to Lydia, a young Slovenian lady, for her help with childcare. Andreas's favorite places in the Institute were the rabbit cages and the scintillation counter, especially the train-like motion of the small bottles.

YALE UNIVERSITY, NEW HAVEN, BACTERIOPHAGE FD

After both of us graduated in Biochemistry, Thomas and I accepted Don Marvin's invitation and moved to Yale University in New Haven, Connecticut. Our financial situation was catastrophic: Our modest stipend from the Max-Planck-Institute for Virus Research from our last month in Tübingen was needed for the move and ran out before any input of finances from Yale University. I remember searching for affordable towels! Such were the times… I am eternally grateful to Don, who helped us with a small loan.

We lived in a small house in East Haven on the Long Island coast. The house was charming but not built for harsh winters. Unexpectedly, such a winter was ahead of us, with a frozen Long Island Sound, windows decorated with the most beautiful ice flowers, and… another child—Michael. In retrospect, it seems as if strong, snowy, ice-cold winters announced children born to Thomas and Barbara; the winter before Andreas's birth, a cold spell had led to frozen lakes in Central Europe. I vividly remember the completely frozen Bodensee, with only a small artificial hole sawn into the ice at the coast near Bregenz to provide a space for the ducks to feed and secure their survival.

According to US custom at that time, a mother had but a few days off after childbirth. Again, I owe a debt of gratitude to Don, who extended this privilege to two or three weeks. This permitted me to screen though *Current Contents* issues en masse to prepare our review on fd (42). Modern scientists cannot imagine how lucky they are having access to all that is published via the internet (and to extended maternity leave!). My work consisted in analyzing the switch from synthesis of double-stranded fd DNA synthesis during the early stages of fd replication to synthesis of single-stranded DNA (e.g., 24).

STANFORD UNIVERSITY, CALIFORNIA, MAINTENANCE OF BACTERIAL PLASMIDS

Originally, we had planned to work in California. Due to Don Marvin's offer that I spend a year of postdoctoral time with him in Yale, the California plan was postponed but not forgotten. In the summer of 1968, we headed west in a rented U-Haul trailer loaded with all our belongings, our kayaks, our au pair, my mother, the two children, and ourselves. The journey took several weeks, and we traveled through many fantastic parts of the United States. On the first part of the trip, from East Haven to Niagara Falls, the driving was shared by Thomas and our Austrian au pair, Marianne, who had just passed her driver's license test in an American automatic car. Our car, which was transported by ship from Europe (with two kayaks on top!), was an old-fashioned, stick-shift model. Thomas assisted from the passenger seat to get into fourth gear, whereupon Marianne could drive through the night without having to change gears! From Niagara Falls westward, we spent several weeks traveling through beautiful landscapes and hiking in national and state parks with our four-month-old baby carried on our backs.

Thomas was to work with Dale Kaiser in the Department of Biochemistry at Stanford University; I was to look for a position upon arrival. This was the deal, as New Haven had been my turn to choose a working environment, and Don Marvin had offered me a paid position. First, we had to find a place to stay; for the first few nights, all six of us camped at a nearby campsite surrounded by giant redwood trees. It was fairly easy at that time (and with the generous and spontaneous help of the Stanford Women's Club) to find a house and fill it with all the necessary furniture and household items within a day! It was a little more difficult to find a laboratory where I could perform postdoctoral work. At first, of course, I tried the famous Department of Biochemistry—after all, this place hosted great scientists, such as Arthur Kornberg, Paul Berg, Dale Kaiser, Buzz Baldwin, Ron Davis, Dave Hogness, George Stark, and others. But… "No women"! This was 1968. So I went one floor down and was welcomed by David Korn, a medical pathologist with a hobby in bacterial replication and maintenance of bacterial plasmids. My work, funded by a National Institutes of Health fellowship, involved the isolation of mutant *Escherichia coli* bacteria unable to maintain plasmids at elevated temperature. I thoroughly enjoyed my work and could be seen in the laboratory even late at night. Together with other women scientists in this laboratory, I was graciously permitted to attend the literature seminars of the Biochemistry Department. I remember a lecture by Paul Berg about plasmids. To demonstrate his theme, he wore a chain of

plastic pearls (these were hippie times!), which he could change from a supercoiled form to an open, circular form.

Altogether, our time at Stanford was a wonderful mixture of scientific excitement and hard work, intensive family life with our two children and our au pair, and travel to many places in the United States and Mexico and island destinations. We tremendously enjoyed visiting Native American Indians, watching their dances, and admiring their artwork. The feeling of personal freedom that marked this time—due to the hippie atmosphere, the antiwar demonstrations, and the (in retrospect) lack of heavy responsibility during student and postdoc days—was never to be experienced again.

As if to put off the onset of "responsible adult" life with (semi)permanent positions, children at school, and financial constraints, we opted to return to Europe via continents west of North America. Thomas and I traveled for three months from California to Hawaii, the Fiji Islands, New Hebrides, the Solomon Islands, New Guinea, the Philippines, and Nepal (via Hong Kong and Thailand), returning eventually to Vienna via Afghanistan, Moscow, and Poland. Our longer stops were in New Guinea, with a trip down the Sepik River, and in Nepal, where we trekked to Annapurna and Dhaulagiri. In Vienna, these selfish parents rejoined their boys, in the care of grandparents during our tour of two-thirds of the world. After a family holiday, we settled down to serious life in Basel, Switzerland.

BASEL BIOCENTER: LAMBDA PACKAGING

Before leaving Germany, Thomas had secured a group leader position with Eduard Kellenberger at the Biocenter in Basel, Switzerland. With his vision for the future of science together with his strong influence on politicians and scientists in both academia and industry, Eduard had established a soon-to-be world-leading institution. Navigating my private and scientific life in Basel at that time was not easy. To give some context, note that women were granted the vote in Switzerland only in 1971! In Swiss society at that time, help with childcare for preschool-age children was almost nonexistent and acceptance of female researchers in institutions, such as the Biocenter or, later, the Friedrich Miescher Institute (FMI) (see below), was not usual practice. However, I took the view (at least I have come to take the view in retrospect) that anything I could not achieve was absolutely my own fault, and nobody else was to blame. As with previous obstacles, I viewed difficulties as challenges to overcome and the fight against them to result in improved strength.

Work in the Biocenter focused on analyzing the structural and genetic basis of the morphogenesis of bacteriophage lambda, which fit well with the work my husband Thomas had carried out in Stanford with Dale Kaiser. Thomas and I joined forces to unravel the intricacies of this process, and his instinct for structure and my instinct for genetics turned out to be complementary and very productive. In addition, the friendly and helpful infrastructure of the department run by Eduard and his wife Cornelia (Cok) was key to the success of our research. Most of Eduard's department was engaged in elucidating the structure and assembly pathway of bacteriophage T4. Collaborations with in-house electron microscopy specialists encouraged Thomas to analyze the structure of mature lambda particles and assembly intermediates. Especially intriguing was the as-yet-unexplained wild mixture of lambda particles of various sizes, full and empty (of DNA). Particles called "petit lambda," devoid of DNA, were at the time considered to be byproducts of assembly.

To understand the assembly of "our" bacteriophage, in vitro complementation experiments were performed. At its simplest, in vitro complementation involved mixing lysates of head- and tail-defective mutants and looking for plaques. More interesting were analyses of mutants that

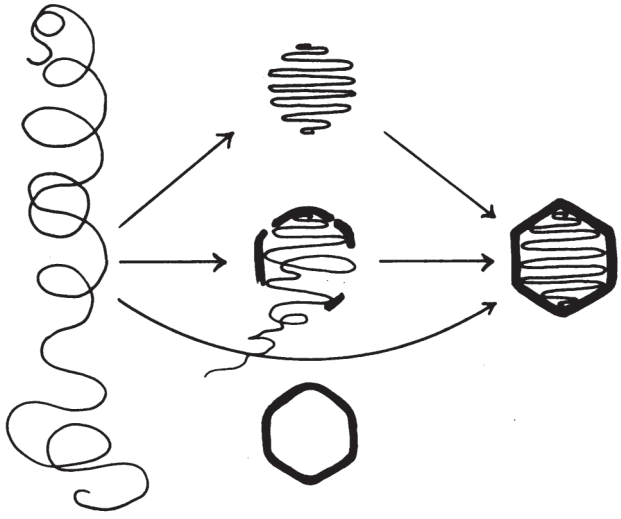

Figure 1

Models for condensation and packaging of DNA (reproduced from Reference 23).

did not produce mature heads but just presumptive precursors. We traced the function of various head proteins back to very early stages to a small, empty head—petit lambda!! We had arrived at the up-to-then elusive stage of DNA packaging. A much-debated question at that time was: Do proteins form a bacteriophage head assembling around a precondensed DNA, or does DNA enter a preformed capsid/procapsid? A third possibility was that DNA and head proteins might coassemble (23) (**Figure 1**). Purified small lambda particles mixed with lysates lacking the main head protein yielded infectious bacteriophage, and lambda DNA isolated from mature particles could be packaged in vitro. These studies thus demonstrated that lambda DNA could be filled into empty particles that were smaller than mature particles (23). Subsequent work demonstrated that expansion of the prohead (petit lambda) to the mature size happens concomitantly with DNA packaging (29).

This was an incredibly exciting time: The age of cloning was sweeping with explosive energy over us scientists but also reaching nonscientists. Hope, anxiety, curiosity, and total refusal were among the then-popular responses to this scientific revolution. Early cloning experiments were performed in *E. coli*, and the first vectors were plasmids. However, two visionary pioneers in bacteriophage lambda genetics—Noreen and Kenneth Murray from Edinburgh—soon devised phage vectors. On a visit to Werner Arber at the Biocenter, Ken suggested comparing the efficiencies of in vitro packaging to another method of rendering recombinant DNA infectious, as proposed by Sydney Brenner and Thomas Hohn: that of making bacteria competent for uptake of DNA by treatment with $CaCl_2$. Ken proposed that he and I undertake this comparison, working side by side. I will never forget those days of working together with Ken on my little bench in the Biocenter in December of 1975, having the most interesting discussions on science and life. I soon realized that Ken saw the future of genetic engineering as very bright and important—a view that, I must confess, was not yet mine. To further our research, Werner Arber allowed us to conduct experiments to compare efficiencies of DNA packaging (in several research groups) as part of a European Organization for Molecular Biology (EMBO) course he organized on recombinant DNA. The packaging method turned out to be far more efficient than direct transformation. We

submitted the results to *Nature*, whose editors decided that the story was not of general interest—a view not shared by the editorial board of *PNAS* (25).

My own cloning experiment employing the packaging system is, in retrospect, a simple story. Costa Georgopoulos, then in Geneva, had identified a function from *E. coli* necessary for lambda morphogenesis, and we decided to isolate the corresponding gene. We cloned random fragments of *E. coli* DNA into a lambda vector and plated the resulting bacteriophage mixture on *E. coli* that was defective for the host function. The few plaques that appeared all contained a fragment consisting of the *GroE* gene (13). Only several years later did it become clear that the GroE protein belongs to the family of chaperones.

Ken Murray convinced EMBO to organize a tour for me to visit several European laboratories devoted to genetic engineering. I remember (in that pre-Euro age) traveling from place to place, changing mid-air the money in my purse from Swiss Francs to British pounds to Swedish Krona to French Francs to German D-Mark and back to Swiss Francs. During my time in Cambridge, I had two extraordinary experiences. The first was to visit Sydney Brenner and give a seminar in his department. I was housed in the oldest Cambridge college, Peterhouse. When I arrived in the breakfast room, the newspapers of the gentlemen sitting there went up so as to avoid eye contact with this unwanted individual. The other—very lovely—experience was walking over the lawn of the college with Sydney Brenner; this was the privilege of professors and their guests only, and Sydney exhibited such happy joy to share it with me, with a boyish smile on his face—small things can be so important! The next stop was to visit Noreen and Ken Murray and give a talk in Edinburgh. This trip included a marvelous hike to the mountain Ben Lawers in snow and sun and storm and memorable views of the surrounding lakes and mountains. I vividly remember the electrical atmosphere provoking a very strange feeling. On the way, we saw thousands of sheep and what I, unaware of their real name, termed "Angoracows"—Scottish highland cattle. Highlights were my discussions with Ken and Noreen Murray and their students and the incredibly warm welcome at their home. My scientific and personal relationships with Noreen and Ken lasted until their premature departures from this world.

Next stop on the tour was Stockholm, Sweden, to visit Giuseppe Bertani. The program consisted of a seminar, lively discussions with group members, and visits to the town with its Vasa ship and Skansen exhibiting medieval Sweden. Via London I moved to the next adventure—Paris. Giorgio Bernardi spoiled me with exciting discussions and exquisite French food (coquilles St. Jacques provençales—I found it marked in my notebook!). Giorgio very proudly showed me his P4 laboratory—a place in which experiments with the then-imagined highest potential risk could be performed. In Paris, I also visited colleagues Josette Rouvière-Yaniv and Moshe Yaniv, who I knew from my days in Stanford.

The last destination of my EMBO seminar tour was Heidelberg, Germany, to visit Hartmut Hoffmann-Berling, the discoverer of bacteriophage fd—the subject of my PhD thesis. Among the colleagues reencountered on this visit was Don Marvin, with whom I had studied the replication of this small bacteriophage, and Heinz Schaller, a friend and colleague from Tübingen.

Lambda DNA packaging became an established protocol in the cloning of recombinant lambda DNA. As a consequence, I was invited to several cloning courses in Basel, Paris, and Heidelberg. A very useful side effect was that I learned other techniques of cloning, hybridization, gel analysis of restriction fragments, and proper handling of radioactive compounds—all of which were immensely useful later on.

By this time (1981), institutions outside of Europe included DNA packaging in their teaching programs; courses were held to introduce these new methods to students also in India and Pakistan. By mere accident, while receiving a request for my packaging strains, I was made aware of a course to be held in Varanasi, India, and led by Ahmad Bukhari, Joe Sambrook, and Anna

Marie Skalka. Over the phone, my wide and interested eyes must have been "visible" to Anna Marie, who had collaborated with us before (7). The next day she asked me to join the course in Varanasi—an Indian city of special scientific and religious importance. The organizers (Americans, with the enthusiastic local aid of Maharani Chakravorty) managed to assemble a group of students, half from India and half from surrounding countries, to attend the first-ever event to teach modern biotechnology in India. It was an incredibly interesting experience to see how eager this group of international scientists was to learn. Technically, it was far from easy, as the American course organizers themselves had to bring, by air, heat-sensitive material, such as bacterial strains (including the very sensitive packaging strains) and radioactive compounds through New Delhi airport. A special challenge was the frequent interruptions to the electricity supply, causing the centrifuges to stop. I remember a procession of students going from one building to the next with buckets of ice, holding burning candles and Eppendorf tubes full of some important stuff, in the dark of night. Eppendorf tubes, by the way, were very rare in India and were reused—luckily this was pre-PCR. Personally, this course was of special value to me, as the company of students from the many countries surrounding India gave me wonderful insights into the culture, educational background, and vision of their countries of origin. With the Indian ladies, I also learned the art of dressing in saris; they came to my room and taught me—an education surmounted by joyful laughter.

Anna Marie was also invited to teach in Pakistan and invited me to join her in Faisalabad—again an interesting and exotic place for us. Our journeys back home were out of Karachi, where delayed flights provided the invaluable experience of spending a few days with Pakistani scientist Mumtaz Jehan, to cook with her, to attend a seminar with her, and to thoroughly enjoy our free time together. Anna Marie recounts her time in India and Pakistan in her review of her life as a scientist—a very interesting read (65).

BASEL BIOCENTER: COSMIDS

A critical step in lambda DNA packaging is recognition of the cohesive end sites (cos) of the DNA by terminase enzyme protein A (20). For cloning in lambda, all phage functions (amounting to ~40 kb) were necessary because plaque formation was needed. The question then was, For biological detection, would a plasmid containing just a cos site do? With perfect timing for answering such questions, John Collins from Braunschweig visited the Biocenter and gave a seminar on the *E. coli* plasmid ColE1. John and I entered into lively and fruitful discussions in which it became apparent that he needed to clone large fragments of DNA, and I suggested exploiting lambda packaging of plasmids containing a cos site. John got excited, I got excited, and we established a collaboration. The only problem was that I had no idea how to handle plasmids, and John was not (yet) an expert in lambda packaging in vitro. However, these mutual deficiencies developed into mutual complementation of expertise. The third and fourth parties involved were the official German and Swiss mail systems. To render it packageable, the DNA of a cos-containing plasmid had to be extended by cloning large DNA fragments into it. John performed the ligation reactions and sent them to me in Basel via the German post. I performed the packaging reactions and transfected *E. coli* with the resulting hybrid plasmids. Individual colonies appearing on selective medium were suspended in buffer, transferred to Eppendorf tubes and sent, via Swiss mail, to Braunschweig for analysis. Back came an enthusiastic letter, dated August 8, 1977, confirming the detection of large cos site– and *E. coli* DNA–containing plasmids. The end of the letter reads: "Anyway tonight the toast will be packageable plasmids: "COSMIDS"". The final story is published in Collins & Hohn (5). An extended review of early developments and uses of the cosmid cloning system can be found in "Ten years of cosmids" by Hohn & Collins (22).

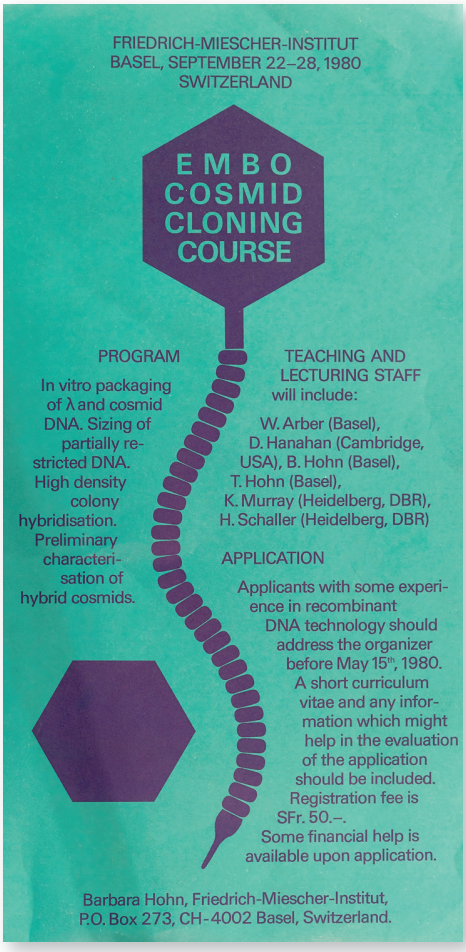

Figure 2

Poster for the European Organization for Molecular Biology (EMBO) Cosmid Cloning Course, 1980.

Cosmid cloning was one of a few methods available to clone large DNA inserts because the in vitro packaging reaction selected for full lambda heads—a total of 50 kb. The first ever EMBO course hosted by the FMI—the EMBO Cosmid Cloning Course—was held in September 1980 (**Figure 2**). Teachers included pioneering scientists from previous courses. Students came from all over Europe but not beyond; only scientists from EMBO member countries were accepted. When Bonni Reichelt from the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) applied, I had to refuse but tried to be polite by answering her letter with something like, "….but I could organize a course in Australia." She asked her supervisor, Liz Dennis, whether this was possible, and, according to Bonni, Liz said, "of course!" Getting to know Liz roughly a year later, Thomas and I realized how she operated: full of energy, full of charm, full of positive attitude—nothing was impossible to her. Bonni had received some training in cosmid cloning in our laboratory and had introduced the relevant techniques to Canberra. After a week of preparations, Liz, Thomas, Bonni, and I led the course with a group of students from all over Australia.

The head of department, Jim Peacock, took great interest in the course, and thoroughly spoiled me and Thomas with invitations to his home, where we were introduced to his friend, a yellow-crested cockatoo.

BASEL FRIEDRICH MIESCHER INSTITUTE: CAULIFLOWER MOSAIC VIRUS

At the end of 1978, our term at the Biocenter ended. It was not at all easy to find a place as a research scientist in or near Basel. It would have been difficult to move our two sons, then 10 and 15 years old, to another school and community. However, Thomas was successful in his application for a group leader position at the FMI. This research institution, founded in 1970 and funded by Ciba-Geigy, was (and is) dedicated to fundamental research. Until the turn of the millennium, it housed researchers in both animal biology and plant biology. Following the merger of Ciba-Geigy with Sandoz, when the company changed its name to Novartis, plant-related research at the FMI ceased. The FMI is now named The Friedrich Miescher Institute for Biomedical Research and continues to conduct research at the forefront of fundamental science.

Even before we started our research at the FMI, it became clear that its plant researchers, especially Ingo Potrykus, were very interested in collaborating with us molecular biologists—it has to be remembered that the new field of molecular biology and the very new advances of molecular cloning were lacking at the FMI at that time. Due to the foresight of Kaspar Winterhalter, then a group leader at the FMI, scientists with a background in molecular biology and cloning were recruited. Thomas, with his interest in viruses and a vision for developing gene technology in plants, searched for a suitable plant virus. In Strasbourg, France, the director of the Institut Biologie Moléculaire des Plantes, Léon Hirth, and his coworker Geneviève Lebeurier were exploring the newly isolated cauliflower mosaic virus (CaMV), which infects and damages turnips and related plants. Now it became obvious that my (still humble) expertise in cloning, as learned in various courses, was of fantastic advantage. However, we realized that our skills in genetic engineering were not sufficient. Consequently, we arranged short (three-month) mini sabbaticals in San Francisco for Thomas and in Stanford for me. Housed in these two different places, we gained critical insights into cutting-edge technology. This time, I reached the holy grail, the Biochemistry Department of Stanford University, where scientists whispered "cosmids" after me in the hallways—apparently this was my entrance ticket. Times had changed greatly and minorities were also accepted. I ended up in the laboratory of Ron Davis, the then-Mecca of gene technology, and was introduced to yeast with all its tricks and treats. Restriction enzymes and the like were not commercially available at that time and each laboratory in the Biochemistry Department was responsible for producing one particular enzyme. These enzymes were kept in unlabeled tubes, the contents known only to the inhabitants of the laboratory that produced them. As a visitor I had access to all enzymes, an immense advantage that others were eager to exploit: "Barbara, could you please let me know whether enzyme XYZ is available and in which lab?" I think only late at night—and we used to work late—I gave in, but not in all cases… Anyway, after three months of intensive training, we returned to Europe but not without a detour to Alaska with our boys.

Back in Europe, real life started and CaMV DNA had to be cloned. Luckily, while collaborating with Susumu Tonegawa at the Basel Institute for Immunology on his famous immunoglobulin switching (38), I had access to their containment facility. At that time, the FMI still lacked such a laboratory, which was thought to be required for cloning of eukaryotic viral DNA. Geneviève brought, in rather big glass tubes, purified CaMV DNA. The complete sequence of this viral DNA—established in a tour de force that amazed not just plant scientists (10)—allowed rational selection of enzymes for cloning the whole viral DNA, and a restriction map was established (27).

Of particular importance was our finding that the cloned CaMV DNA, separated from the vector DNA, was infectious when rubbed together with an abrasive onto turnip plants (37). We also cloned two fragments of CaMV DNA cleaved with *Bam*HI, each in its own vector. Coinoculation of turnip plants with the larger fragment, Bamone, and the smaller one, Bamino, each released from its bacterial vector, resulted in CaMV symptoms, demonstrating in vivo ligation of the two fragments. Also, in vivo homologous recombination (HR) could be shown by coinoculation of two clones of CaMV integrated into bacterial plasmids at different unique restriction sites and recovery of infectious virions (36). This success initiated my interest in HR, which later extended to recombination in the context of chromatin.

The CaMV story continued mainly in Thomas's laboratory—I moved to my own laboratory around 1984, although with limited funding. This actually turned out to be a benefit as I tried to collaborate as much as possible and to use the infrastructure of other groups to support my small laboratory. The CaMV team, meanwhile, made the milestone discovery that this virus is a pararetrovirus (53). Consistent with this interpretation was the result of my initiative to test whether an artificial intron, squeezed into an unessential region of CaMV DNA, would persist in replicating viruses: It did not. Clean splicing led to excision of the intron from the viral RNA and to spliced versions of viral progeny (21).

In the following 20 years or so, CaMV and related viruses continued to be exploited in the laboratory of Thomas at the FMI and then at the Botanical Institute of the University of Basel as a model for cloning DNA in plants, for exploring unusual translation mechanisms, and for studying gene silencing (26, 28).

BASEL FRIEDRICH MIESCHER INSTITUTE: *AGROBACTERIUM TUMEFACIENS*

I opened my own laboratory with several ideas in mind. Studies on CaMV replication continued, but concentrating on other new developments in plant genetic engineering became my priority. Two related lines of research emerged: plant genetic transformation, in collaboration with the laboratory of Ingo Potrykus; and research into the mechanisms of genetic transfer of DNA to plants by the bacterium *Agrobacterium tumefaciens*. It had just been discovered that plants could be transformed by *A. tumefaciens*, but Ingo, later the hero of Golden Rice (54), and I wanted to test pure DNA for transformation. Jerzy (Jurek) Paszkowski, an enthusiastic coworker of Ingo's, after having been introduced to molecular biology in my laboratory, managed to construct a vector and to transform tobacco cells with free DNA. The recovery of fertile transgenic plants caused enormous excitement (50a)!

With the arrival of my first PhD student and future postdoc, Zdena Koukolíková-Nicola, I became the fortunate advisor of a very special research scientist; Zdena had a vision for exciting questions and an immense sense of curiosity. It was her idea to retrieve transferred DNA (T-DNA) integrated into plant DNA by cosmid cloning! A few words to explain the system: *A. tumefaciens*—a plant pathogen causing tumors on susceptible plants—contains a plasmid termed tumor-inducing (Ti) plasmid. Part of it, the T-DNA delineated by so-called border sequences, can move to plant nuclei and integrate into plant chromosomal DNA at random positions (reviewed in 12, 30, 49). It was Zdena's wish to invite Marc Van Montagu (73), who is, together with the late Jeff Schell, a hero of *A. tumefaciens* research, to present an introduction to this fascinating interaction of bacterium and plant. There were then—and still are—many unresolved issues: Where in the plant genome does T-DNA integrate? What is the mechanism of this integration? Can we convince the T-DNA to integrate at locations of our choice? How is the T-DNA removed from the Ti plasmid? How and in what form does it travel to the plant cell? How does it find the nucleus?

For recovery of integrated T-DNA and adjacent plant DNA sequences, the T-DNA was endowed with a cos site, a bacterial origin of replication, and selectable markers for both *E. coli* and plants. This arrangement was expected to permit the isolation of T-DNA/plant DNA junctions through lambda DNA packaging. However, upon analysis of bacterially residing T-DNA before transfer, a large number of cosmids containing border–border junctions could be detected. This meant that the T-DNA could free itself from the Ti plasmid and that the isolated T-DNA molecules could be intermediates in T-DNA transfer. This work was complemented by experiments from the group of Patricia Zambryski, and we published together (32). At that time, we interpreted the rescued plasmids as circular intermediates in T-DNA transfer to plants. However, later work showed that true intermediates are linear and single-stranded versions of the T-DNA. Zambryski's group identified single-stranded versions of the T-DNA in bacterial cells induced for transfer to plants (67), while a genetic trick using T-DNA as a recombination substrate led us to the same conclusion (69). An elegant experimental scheme, devised by Nigel Grimsley from my group, employed "good old" CaMV DNA that had been cloned into T-DNA in *A. tumefaciens* to examine the junctions of resulting viruses; this suggested that a transfer intermediate must be linear and that filler DNA is frequently integrated between the two joined border sequences—a characteristic akin to integration of T-DNA into chromosomal DNA (1). These studies led to the conclusion that T-DNA transfer to plants must have derived from conjugation—the transfer of bacterial DNA to bacteria. It remains unclear to this day how T-DNA genes, which are only poorly expressed in their bacterial surroundings, have acquired eukaryotic expression signals to permit genes located on the T-DNA to be active in the plant nucleus.

Our work then focused on virulence proteins VirE2 and VirD2, which play very special roles in the transfer of T-DNA to plants and its integration into chromosomal DNA. Both are transferred to plants; VirE2 as a protein and VirD2 attached to T-DNA. VirE2 proteins bind to single-stranded DNA in a sequence-unspecific manner (15). In a milestone paper, Vitaly Citovsky in Patricia Zambryski's laboratory demonstrated that this protein, when expressed in plant cells, complemented to virulence *A. tumefaciens* strains lacking VirE2 altogether, thus clearly showing that the activity of VirE2 proteins for T-DNA transfer resided in the plant cell recipients (4).

The virulence protein VirD2 is a site-specific endonuclease, which cleaves both border sequences at a precise location. As a consequence, VirD2 protein becomes covalently attached to the 5′ terminus of one strand of T-DNA, rendering the protein–DNA complex resistant to 5′ to 3′ exonucleolytic attack (8). One of the ensuing questions was, What could be the function(s) of VirD2 protein in the plant? Inspection of the protein sequence turned out to be illuminating. It contained two nuclear localization sequences (NLSs)—quite amazing for a protein produced in a prokaryotic organism! Of course, a possible function had to be tested: Bruno Tinland fused the NLSs to marker genes and found that the hybrid proteins were directed to the nuclei of yeast and tobacco cells (70), while Luca Rossi tested the NLSs for their importance in transfer and integration of T-DNA in the context of transformation. The C-terminal NLS was of critical importance, whereas deletion of the N-terminal NLS had no effect (60). This was not enough for us curious molecular biologists; we needed to see T-DNA and protein molecules in action. Alicja Ziemienowicz, a Polish postdoc who had been well trained in biochemistry, undertook the purification, labeling, and assembly in vitro of bits and pieces of the T-DNA complex—DNA, VirD2, and VirE2—and studied nuclear import in tobacco and HeLa cells—the latter of which permitted the test of import factors. Nuclear import of single-stranded T-DNA turned out to be dependent on the virulence proteins VirD2 and VirE2 and import factors known for mammalian systems (75). Use of the tobacco cell–derived nuclear import system allowed us to conclude that the NLS-dependent function of covalently attached VirD2 is absolutely essential, and that, at least for longer single-stranded DNA versions, the presence of VirE2 is required, possibly

as a structural and neutralizing component of the complex (76). These two virulence proteins have thus evolved to lead prokaryotic DNA harboring genes with eukaryotic promoters into a eukaryotic nucleus—a remarkable evolutionary strategy indeed.

A collaboration between the group of Csaba Koncz in the department of Jeff Schell and my laboratory found that the junctions between plant DNA and T-DNA are very precise at the border to which VirD2 is covalently bound, suggesting a special role for VirD2 (45). Also, the junctions of T-DNA with itself, found as inserts in rescued CaMV genomes, are precise at the original T-DNA 5′ terminus to which VirD2 was once bound (1). Even in a mammalian test system, a humanized T-DNA inserted into the HeLa cell genome in precisely the same manner as in plant cells. This depended on VirD2 bound to the mammalian T-DNA and on VirE2 molecules coating the single-stranded DNA (52). With this experiment, Pawel Pelczar could also show that for precise integration both virulence proteins are necessary and sufficient.

A fascinating side story evolved after a phone call from Hawaii: Ryuzo Yanagimachi, director of the Department of Anatomy and Reproductive Biology in Honolulu, Hawaii, expressed his interest in our in vitro transgenesis system. So, "poor" Pawel went to Hawaii to try to inject some constructs as single-stranded DNA coated with VirE2 into mouse sperm. Misbehaving VirE2 protein preferred to aggregate in the injection needles and was soon replaced by the well-behaved and commercially available RecA protein from *E. coli*. The result was efficient transgenesis with improved embryo survival rate (31).

Back to the main story: Another indication that VirD2 is involved in integration was an experiment involving a mutated VirD2 that resulted in aberrant integration patterns (71). However, it was clear that, at least in vitro, VirD2 by itself is not a sequence-unspecific DNA ligase, as would be expected for a T-DNA integrase (77). However, direct biochemical analyses were lacking. You-Qiang Wu tested whether VirD2 would interact with plant DNA ligase I, a prominent candidate for integration, in vitro. Indeed, these proteins interacted, even when bound to single-stranded DNA mimicking a transfer intermediate (Y.-Q. Wu, P. Pelczar, A. Ziemienowicz & B. Hohn, unpublished data). Christopher E. West confirmed the VirD2–plant DNA ligase interaction in a split YFP assay (C.E. West, unpublished data) (**Figure 3**).

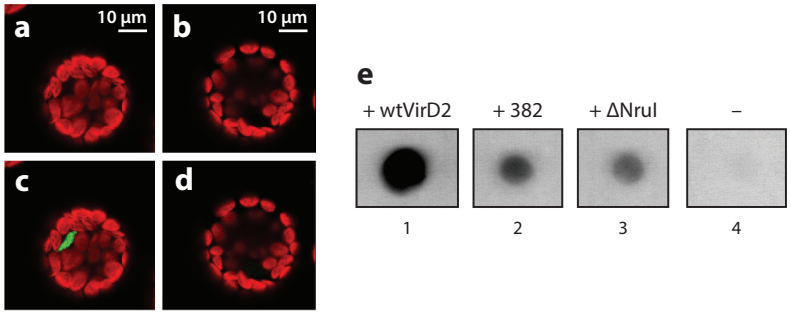

Figure 3

Interaction between *Arabidopsis thaliana* DNA LIGASE 1 (LIG1) and *Agrobacterium tumefaciens* VirD2. (*a*,*c*) Confocal images showing split YFP interaction between LIG1–nYFP and VirD2–cYFP. (*b*,*c*) Control showing no interaction between LIG1–nYFP with cYFP. (*a*,*b*) Chlorophyll autofluorescence. (*b*,*d*) YFP signal merged with chlorophyll autofluorescence (C.E. West, unpublished data). (*e*) Dot blot showing interaction between LIG1 and VirD2. A region spanning the C-terminal nuclear localization sequence (NLS) is required for complex formation. Lanes 1–4, aliquots of 100 ng of *A. thaliana* DNA ligase I, were blotted and overlaid with wild-type VirD2, mutant 382, ΔNruI, and blotting buffer, respectively. Detection of interaction was with VirD2 antibody (Y.-Q. Wu, unpublished data).

Integration of T-DNA can be viewed as a repair process of injured plant DNA. Repair enzymes have been tested indirectly by transforming repair-deficient *Arabidopsis thaliana* plants, with unclear results. Only mutants in DNA polymerase theta were found to be nontransformable (72). This enzyme is proposed to be responsible for integration of the 3′ terminus of T-DNA, while the 5′ terminus is attached to plant DNA in a VirD2-dependent manner (see above). The form in which T-DNA integrates, single stranded or double stranded, has long been a matter of debate. In a commentary on the Pol theta publication, Avi Levy (39) proposed a single-stranded version, conforming to our previous suggestion and our integration model (71). The function of VirE protein seems to be to protect the single-stranded T-DNA by coating this molecule. Indeed, in the absence of VirE2, only severely truncated versions of T-DNAs are integrated, as shown by Rossi et al. (61).

Besides the intriguing issue of the mechanism of T-DNA transfer and integration, the question of the host range of *A. tumefaciens* was also of basic and applied interest. After all, many plants important for food and feed are monocotyledonous and were considered to be outside the host range of *A. tumefaciens*. In collaboration with my husband, we had established with CaMV the procedure of "agroinfection," the name we gave the route by which *A. tumefaciens* carries infectious viral DNA into plants (16). [As an aside: This procedure is used to overexpress proteins of medical interest from genes inserted into viral DNA and transferred to cultured plant cells via *A. tumefaciens*–mediated DNA transfer (41).] Nigel Grimsley, Thomas, and I investigated whether agroinfection would work in maize, but several problems needed to be solved [described in detail in "*Agrobacterium tumefaciens*: From Plant Pathology to Biotechnology" (50, pp. 249–54)]. We did not know whether maize can host *A. tumefaciens*; a clone of the circular single-stranded DNA of maize streak virus could not be shown to be infectious. We did not know which strain of *A. tumefaciens* to use or which part of the plant should be agroinfected. After two years of agroinfection experiments in the P3 laboratory of the FMI and newly equipped with strong lamps, Nigel called me, saying, "Barbara, we have symptoms!!!" (**Figure 4**). After plant DNA analysis assured us that the symptoms resulted from replicating maize streak virus, we concluded that maize is a host for DNA transfer by *A. tumefaciens* (17), and this was shown for many other monocot plants thereafter. The world had to wait another 10 years or so for the establishment of stably transformed rice and maize plants: The large and hardworking group of Toshihiko Komari in Japan tested many parameters until finally achieving success, as described in his recent review (19). In our group, maize agroinfection was exploited by Wen-Hui Shen; the transposable element Ds was introduced into the viral DNA and parameters for its excision were analyzed (e.g., 64).

BASEL FRIEDRICH MIESCHER INSTITUTE: DNA REPAIR

Considering the path of a viral genome from its bed in a T-DNA, on its way to the plant, and its escape from the T-DNA, we envisioned several models: replication out from a T-DNA intermediate or its integrated version or excision by HR from a (partial) dimer of the viral DNA. CaMV agroinfection likely follows a path of transcription and reverse transcription (18). At the same time, my laboratory hosted a group of scientists eager to unravel the genomic recombination route and decided to look at recombination within plant chromatin. As sensitive as the viral system can be, it could not reveal the mechanisms and specificities of chromosomal recombination. Holger Puchta, Peter Swoboda, and Susannah Gal set out to establish a system to study just this—and in doing so set in motion a cascade of research activities across the world. Indeed, the recombination tester lines in *A. thaliana* and in tobacco established by Holger, Peter, and Susannah continue to be used by the international community. Overlapping parts of the β-glucuronidase (*GUS*) gene were transformed into tobacco and *A. thaliana* plants (69). We were completely overwhelmed when the first blue recombination spots appeared on histochemically stained plants carrying the recombination

Figure 4

Symptoms of maize streak virus on agroinfected maize plants. Cover of *Nature*, Volume 325, Nr 7000, 8–14 January 1987, titled, "DNA transfer to maize."

marker (68) (**Figure 5**). However, use of these lines requires carefully controlled conditions (56). Recombination spots were detected in all analyzed organs of *A. thaliana* plants. This experimental system laid the foundation for student and postdoctoral work in my laboratory for many years, following exciting questions on HR frequency and the influence of abiotic and biotic factors on HR in treated plants and their progeny.

Different plants carrying recombination reporter lines exhibited different frequencies of intrachromosomal HR events. These were defined as the number of blue spots related to the approximate number of genomes present per plant. The differences were interpreted to be due to difference in gene activity in different locations of the transgenes in the genome (68). What we did not expect and still do not fully understand is the distribution of HR frequency in a population of genetically identical plants within a population (57). Some of the analyzed lines showed a Poisson-like distribution, indicating that recombination at the respective chromosomal location was random. However, other lines exhibited a different distribution, and plants with either especially many or especially few recombination spots were overrepresented. Moreover, induction of HR by physical or chemical parameters led to a stronger distortion of the distribution of HR frequency (58). A wealth of possible explanations was given by Holger in the discussion of his 1995 *Plant Molecular Biology* paper (57). Could differences in the distribution of HR frequency be due to different states of the recombination locus? Could these differences be due to different expression levels of the recombined sequences even though the 35S promoter, which directs expression of the unrecombined and recombined versions of the *GUS* genes/fragments, is supposed to be

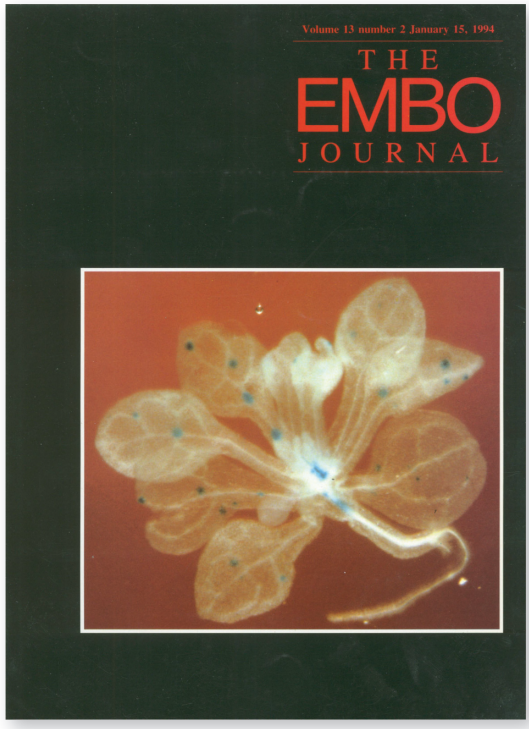

Figure 5

β-Glucuronidase (GUS) spots on histochemically stained plants of *Arabidopsis thaliana*. Cover of *THE EMBO JOURNAL*, Volume 13, Number 2, January 15, 1994. Reproduced with permission from EMBO.

constitutive? At the time of these experiments, visualization of recombination required a lethal histochemical stain, but we did discuss the possibility of using a different assay to test the question of whether the high-recombination and low-recombination plants constitute intrinsic, heritable differences. We discussed the possible meaning of the nonrandom distribution of recombination events: "It seems that these plant populations as a whole have a broader repertoire to deal with their environments" (57, p. 291). Single individuals that recombine more frequently might have better fitness under changing environmental conditions. In the case of somatic selection, the new rearrangement might even be transferred to the next generation, as shown for maize (6) and, in my laboratory, for *A. thaliana* (59). Plants with rare recombination events, on the other hand, might do better under constant growth conditions.

However, what might these results mean for bona fide plant DNA? Parts of *GUS* genes are not known for their relevance to plants. In common with other higher eukaryotes, plants contain numerous repeated sequences in various relative orientations that are therefore valid targets for recombination. Copy number variation as a consequence of recombination is of agronomical importance. Our results on HR in *A. thaliana* and tobacco therefore can be applied to other plants, especially the agronomically important monocotyledonous plants with their large genomes containing huge numbers of repeats, mainly in the form of retrotransposon graveyards. The influences of abiotic and biotic factors (see below) may also be of relevance for sequence repeats in genuine plant genes.

Not surprisingly, various chemical agents and UV irradiation lead to increased rates of recombination (reviewed in 63). An unintentional experimental field condition offered itself in the form of the exploded reactor in Chernobyl, which released large amounts of radioactive compounds. My Ukrainian coworkers at that time, Olga and Igor Kovalchuk, received permission to plant *A. thaliana* recombination tester lines in small plots at various distances from the reactor and found an amazingly close correlation between radiation dose and HR frequency down to very low doses. Thus, a sensitive bioindicator could be established for radioactive contamination (33) and toxic inorganic compounds (35). Since HR requires chromosomal breaks, the introduction of target sequences for site-specific endonucleases in close proximity to the recombining sequences, together with a supply of these enzymes, led to enormous increases in HR-dependent gene targeting. This work by Holger Puchta in my laboratory, and later in his own, demonstrates a breakthrough advance in our understanding and application of HR and gene targeting (55). Another interesting example is a series of experiments using sun simulators with different UV-B doses; elevated rates of recombination were found for both *A. thaliana* and tobacco plants carrying recombination marker genes. The nonexposed F1 and F2 generations still exhibited increased rates of recombination compared with the progeny of untreated plants, at least in one tested line (see below). Of special relevance, from an evolutionary point of view, was the result that the number of germinal recombination events also increased (59).

One surprising finding, at least at that time, was the discovery that treatments with pathogens also led to elevated HR frequencies. In collaboration with Brigitte Mauch-Mani, then at the University of Fribourg, Jan Lucht in my group found that treatment of *A. thaliana* plants with the oomycete *Peronospora parasitica* could stimulate HR (40). This was interpreted as stress-induced elevation of recombination levels, similar to the stress-induced stimulation of transposition shown by Barbara McClintock (46). Infection of tobacco lines carrying HR marker genes (based this time on fluorescence-emitting marker proteins) with tobacco mosaic virus also led to enhancement of recombination in infected and distant leaves, pointing to the systemic spread of a signal other than a replicating virus (34). We suggested that somatic recombination events stimulated by pathogen stress and their transmission to progeny might be involved in the evolution of plant resistance gene clusters and, thereby, of new pathogen resistance specificities.

The availability of recombination tester lines of course demanded application in a mutagenesis-based search for genes involved in recombination. I remember the enthusiasm in the laboratory when student Olivier Fritsch found the first plant with a drastically increased number of recombination events—those are the days one remembers! Of course, very hard work ensued to exclude false positives, to verify the new finding, to identify the gene responsible, and to establish the mechanism of action of the new (for plants) protein. The chromatin remodeling factor INO80 was finally shown to be responsible for the elevated levels of HR in one mutant (11). Centrin 2 was another factor found to be involved not only in HR but also in nucleotide excision repair (47). Those are just two examples of the huge screens we performed; many further candidates await analysis and genetic characterization. The *A. thaliana* chromatin assembly factor Caf-1 was also shown to be active in promoting, directly or indirectly, the rate of HR, as shown in collaboration with the group of Seiichi Toki in Japan (9). DNA replication stress, brought about by a reduced supply of DNA polymerase delta, was also shown to result in increased genome instability, measured as increased HR frequency (62).

Very late in my active research life, even after retirement in 2004, fascinating new findings turned up. Earlier, Gerhard Ries had found while studying the effect of UV on HR that following exposure to UV subsequent generations apparently still "remembered" the stress, exhibiting levels of HR considerably higher than offspring of nontreated *A. thaliana* plants (see above). Here was an example of serendipity! Gerhard had used some seeds without having carefully checked the

content of the sample. Only later, after finding the unusually high HR frequency, did he check the label on the envelope: The seeds in the envelope came from UV-treated plants. His results were published (59) and no one in the laboratory except me was interested in this esoteric result any more. Just to keep me quiet (or happy!), Jean Molinier decided to try to reproduce this experiment using six different recombination lines. Indeed, in all of them, exposure to UV led to elevated HR frequencies in the untreated offspring. The application of peptide flagellin, which was known to induce a pathogenic response in plants, also led to stimulation of HR in the untreated progeny of the two tested lines (48). As interesting as these findings may be, attempts to reproduce them in other laboratories led to contradictory results. The group of Ortrun Mittelsten Scheid found only a "low and stochastic increase in somatic homologous recombination" in nonexposed generations (51), whereas Igor Kovalchuk and his group found transgenerational effects but only to the first generation (3). It remains unclear whether differences in growth conditions or other, possibly unknown factors influence the delicate sensitivity of plants to environmental impacts and their potential to relate influences from one generation to the other. I certainly would have loved to follow these questions further and to have tried to get at the molecular, probably epigenetic, basis of the transgenerational/intergenerational phenomenon we discovered. Of special interest would be the question of whether endogenous plant loci could also receive and transmit a "memory" of what they experience.

As noted above, Jan Lucht and Brigitte Mauch-Mani had shown that inoculation of *A. thaliana* plants with *P. parasitica* led to increased HR levels. In rediscussing these results with Brigitte, I asked the then-unorthodox question of whether she thought that the untreated offspring of infected or primed plants may "remember" their past and may exhibit elevated levels of resistance to pathogens. They did, as Brigitte showed in her experiments (66). Studies from other laboratories also came to similar conclusions, as reviewed in Mauch-Mani et al. (44). Memory of abiotic, biotic, and probably internal influences and stresses seems to be a general feature of both plants and animals.

CONCLUSIONS AND GENERAL CONSIDERATIONS

I consider myself very lucky to have lived through prime times for genetics, gene technology, and molecular plant biology. Knowledge from other institutions and scientists greatly influenced the research of my groups. More importantly, the steady input of ideas from my group members contributed enormously to my scientific output. I tried to keep an open place for my coworkers to develop, and, in turn, I received the merits of intelligent research projects and intense experimentation. Enthusiasm prevailed, at least most of the time, and an open mind for serendipity led to new developments—after all, who would have thought that studying a plant tumor would pave the way to plant transgenesis? I am still in contact with many of my former coworkers; in 2016, I invited them to my home, and we celebrated a fantastic reunion (**Figure 6**).

I was lucky to have been present at Stanford during a breakthrough in gene technology; I had the chance to go to Gordon Research Conferences at which the very first transgenic plants were introduced; I had the privilege of working at the Biocenter in Basel, one of the early breeding grounds for molecular biology; and, finally, I was lucky enough to start my career as a plant molecular geneticist at the FMI in Basel.

I learned from (and contributed to) international courses such as the Indo–Swiss Collaboration in Biotechnology, funding work on pulses, a network assembled by the International Agency of Atomic Energy (for mutagenesis research with scientists from the developing world), and EU-funded consortia on plant DNA repair. I tremendously enjoyed collaborations with several Japanese scientists. All of these experiences broadened my perspective and gave me a chance to

Figure 6

Reunion of former coworkers of Barbara Hohn, 2016.

visit exciting places and develop new scientific friendships. I feel very proud and happy to have been involved, via interactions with the founding director Dieter Schweizer, in the establishment of the Austrian Gregor Mendel Institute of Molecular Plant Biology in Vienna.

A special network for me was and still is a small group of female plant scientists. The exchange of ideas on science, politics, nature, and social and family life was and is of great help, especially in difficult times.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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