Pal Maliga

How did you spend your career?

I graduated with a Masters' Degree in Genetics and Microbiology from Eotvos Lorand University in Budapest, Hungary in 1969. During my studies there, I learned the basics of plant tissue culture. These skills, the ability to initiate sterile cultures from tobacco stem sections and to regenerate entire plants from cultured cells, proved to be extremely useful when I joined the Institute of Genetics, Hungarian Academy of Sciences.

At the time, academic inquiry in Hungary was untethering from the confines of Stalin-era political restrictions, including the influence of his favorite pseudo-scientist, Trofim Denisovich Lysenko, who rejected Medelian-Morganian genetics on ideological grounds. To help with the recovery, the Hungarian Academy of Sciences established The Biological Research Center (BRC) in Szeged, to create a new home for cutting edge science, and scientists were given carte blanch to come up with great ideas. My first appointment as Research Assistant was in the Institute of Genetics, one of the Institutes in the BRC. One day, while reading Science in the library (this was at the time when "Western" literature, such as Science was not generally available), I came across a paper by Ruth Sager, who wrote about isolating streptomycin resistant mutants in Chlamydomonas, a unicellular alga. I thought I could use tobacco cell culture to perform this mutant screen in flowering plants. The

selection worked instantly, and we isolated the first maternally inherited antibiotic resistant mutant in a seed plant. The finding was significant enough to justify publication in Nature New Biology in 1973, and it instantly established my credibility. This early success was the foundation for my life-long interest in organellar genetics. In 1972, I earned a PhD in Genetics and Microbiology from Jozsef Attila University in Szeged, Hungary, working on the isolation and characterization of mutants in cultured cells.

In 1973-1974, I added new experimental tools to my skillset, protoplast culture and fusion, thanks to my friendship with Wilf Keller, who's postdoc was overlapping with my stay in the laboratory of Georg Melchers in Tubingen, Germany. In 1974 I moved back to Szeged as Group Leader/Section Head at the Institute of Plant Physiology. My laboratory continued refining mutant selection and exploited protoplast fusion to study organelle sorting and organelle genome recombination. We also developed new diploid models, *Nicotiana sylvestris* and *Nicotiana plumbaginifolia*, that were almost as amenable for tissue culture as *N. tabacum.* We set up a genetic screen that led to the demonstration of interspecific chloroplast genome recombination in fused protoplasts of *N. tabacum* and *N. plumbaginifolia*; the results were published in PNAS in 1985. This finding suggested that plastid genomes in chloroplasts can be engineered like mega-plasmids in bacteria. At the time I was well recognized for the contributions of my group to cell biology, but by the end of the 1970s it was increasingly clear that the future would require molecular understanding of plant functions. Given my background, I became interested in chloroplast genome engineering. This was ahead of its time, since chloroplast transformation in *Chlamydomonas reinhardtii* was reported only in 1988, and I could not break this barrier in a flowering plant till 1990.

Around the turn of 1980, plant cell culture expertise was in short supply in the US. I had multiple offers to trade expertise in cell culture for training in plant molecular biology. I started with Mary-Dell Chilton at Washington University in St. Louis, and in 1983 I joined Advanced Genetic Sciences (AGS), a biotech startup in the San Francisco Bay Area, as Research Director for Cell Biology. At this time, AGS was a very exciting place to work; it had some of the best plant scientists at an early stage of their scientific careers. This is where I started

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to work on chloroplast engineering. My wife Zora Svab moved from Hungary with our two small children to join me at AGS and start what would become the lifelong support of my research. The approach we used was selection of chloroplast-containing transgenic cells lines in tissue culture by streptomycin or kanamycin resistance, based on the expression of transgenes engineered for chloroplast expression. The problem was that we could not find the rare transplastomic clones in the sea of nuclear transgenic events. In 1988, AGS decided that the chloroplast engineering project was unlikely to succeed and closed it down. I was ready to move on and accepted an offer from Jo Messing of a Full Professorship in the Waksman Institute of Microbiology at Rutgers University, in Piscataway, NJ. AGS/DNAP was very supportive during my time in the company, and in transition to academia.

As a member of the Waksman Institute my teaching load was very light, which enabled me to quickly restart the research I left behind at AGS. Help from my wife Zora was critical for the rapid restart at Rutgers, and in two years we were able to set up a system for plastid transformation in tobacco. We used selection for 16S rRNA-encoded spectinomycin resistance, which helped avoid frequent recovery of nuclear transgenic events. The next step was to identify a suitable chimeric plastid marker gene. To help discriminate between research at AGS and at Rutgers, we fortuitously picked the *aadA* (aminoglycoside-3"-adenylyltransferase) spectinomycin resistance gene instead of the streptomycin phosphotransferase gene we used at AGS. Selection for spectinomycin resistance encoded by *aadA* yielded an abundance of transplastomic events and a relatively small number (10%) of nuclear transgenics. Chimeric *aadA* genes are still the most frequently used chimeric genes as selective markers for plastid transformation.

With the confidence gained by successfully transforming tobacco plastids, we initiated a new project in *Arabidopsis thaliana*. At the encouragement of George Redei, with whom I stayed in contact over the years, Arabidopsis had been on my list of targets for a while. The results were very disappointing though, because the transformation efficiency of Arabidopsis was extremely low, an enigma that remained unexplained for twenty years. But that mystery was solved when David Meinke's group showed that duplication of a fatty acid biosynthetic pathway in Arabidopsis eliminates the need for plastid translation, the driving force that is essential for selective enrichment of transformed plastid genomes. When using genetically engineered Arabidopsis plants as targets in 2017, we obtained Arabidopsis plastid transformation efficiencies comparable to those of tobacco. The problem of regenerating fertile plants from cultured Arabidopsis cells is still an impediment to the general use of this technology. Right now (2021), we are engaged in reengineering Agrobacterium to deliver T-DNA to plastids, so that we can use floral dip transformation of plastids in germline cells instead of transforming the plastids in tissue culture cells. This is a project we already tried with Barbara Hohn in the early nineties, but without much success. The project at Rutgers was carried out by Peter Hajdukiewicz, who constructed the pPZP Agrobacterium binary vectors for the collaborative project on plastid transformation. pCAMBIA and GATEWAY Agrobacterium binary vectors, which are widely used today, are derivatives of the pPZP plasmids. Zora's training in Rhizobium genetics was very useful in the execution of the vector project. Besides Arabidopsis, we tried our hands at rice multiple times and are ready to go back if the opportunity arises.

Over the years, my research group at Rutgers made discoveries that shed new light on the genetics and molecular biology of plastids by knocking out genes from the plastid genome, including *rbcL*, *psbA*, *ndh* and *clpP*. We also knocked out subunits of the multi-subunit plastid RNA polymerase, thereby revealing a division of labor between the multi-subunit, plastid-encoded RNA polymerase (PEP) and the single-subunit, nuclear-encoded plastid RNA polymerase (NEP). We used *in vivo* dissection of plastid promoters that until then were studied only *in vitro*. We characterized multiple NEP and PEP promoters, including promoters upstream of the plastid ribosomal RNA genes, which are the best choices to drive transgene expression. Lori Allison, who played

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a significant role in the early phase of this research, brought her yeast RNA polymerase expertise to the lab. The promoters we characterized are widely used in biotechnological applications.

My lab has played a significant role in establishing the rules of mRNA translation in plastids, the foundation for expression of recombinant proteins in chloroplasts. In the 1990's, we addressed the importance of codon usage for translation. In collaboration with Calgene, we were able to express an unmodified high-AT-rich bacterial gene encoding the *Bacillus thuringiensis* insecticidal protein in chloroplasts; this circumvented the existing Bt patents based on nuclear expression of codon-optimized high-GC synthetic genes. Later chloroplast experiments that expressed a tetanus toxin fragment confirmed chloroplasts can efficiently translate both high-AT and high-GC rich mRNAs. A "by-product" of this research arranged by Peter Nixon, our collaborator at Imperial College in London, UK, used the chloroplast-expressed protein for mucosal immunization of mice to induce protective levels of TetC antibodies. A recent project with Alice Barkan has shown that regulated protein expression can be obtained using a cognate protein binding site upstream of a plastid transgene and an engineered PPR10 RNA binding protein expressed in the nucleus. We are now designing polycistronic expression units with predictable protein output for chloroplasts and are using regulated protein expression for production of oral vaccines.

The chloroplast biotech toolkit was rounded out by the excision of marker genes using site-specific recombinases, a project that was supported by the USDA Biotechnology Risk Assessment Research Grant Program for several years.

My story would not be complete without mentioning my involvement with the New York Hungarian Scientific Society, and the Association of Hungarian-American Academicians (AHAA). I am involved in the AHAA as Foreign Member of the Hungarian Academy of Sciences. The government in Hungary is anti-GMO, and I made it my mission to help my colleagues in Hungary by pointing out the opportunity cost of not pursuing genetic engineering whenever I can.

By now I have spent more than 30 years at Rutgers. I love the location, the unbeatable combination of the cultural offerings of New York City, the proximity and charm of the Jersey Shore, and the closeness of big airports (EWR, JFK) with non-stop flights to many international destinations and good connections to the ski resorts in Utah and California.

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

My most important contribution to plant science is development of chloroplast genome engineering in flowering plants. To put this in a broader context, working with flowering plants has its unique challenges as compared to working with other photosynthetic organisms. If you work with photosynthetic bacteria or unicellular algae, you can always select for restoration of photosynthetic competence. This approach is not feasible in flowering plants, because by the time a plant cell culture becomes photoautotrophic, accumulated genetic changes (somaclonal variation) prevent plant regeneration. Our trick was to find a short-term tissue culture protocol that provides a selection pressure favoring the maintenance of rare transplastomic events (note that there are thousands of ptDNA copies in a plant cell) that are compatible with regeneration of fertile plants.

The system for plastid genome engineering has evolved over more than a decade. The most important discovery was that selection for resistance to inhibitors of protein synthesis is suitable for selective enrichment of plastid genomes in photoheterotrophic culture. The second important discovery was that plastid genomes in fused chloroplasts behave as bacterial mega plasmids, suggesting use of homologous targeting by flanking sequences as the principal approach. Based on these observations, we designed plastid vectors in which a marker gene is flanked by plastid DNA sequences. The two milestone papers reporting introduction of a foreign DNA into the plastid genome, initially at a low, then later at high frequency, were published in 1990 and 1993. The hero of both projects was my wife Zora. Most of our research is carried out in tobacco, which

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has become the model species of chloroplast genome engineering in flowering plants. In the meantime, using a variant of the tobacco protocol, others added several species, including potato, soybean and lettuce, to mention only the most important crops.

The technology we developed at Rutgers is widely used for chloroplast engineering. We started up new research fields through collaborations. A collaboration with Steve Gutteridge at DuPont on Rubisco engineering was an early foray into engineering the plastid photosynthetic machinery. Engineering of Rubisco in tobacco chloroplasts today blossoms in the laboratories of Spencer Whitney and Maureen Hanson. Our early research on RNA editing in chloroplasts was carried on by Maureen Hanson. Ralph Bock, who started his career by studying RNA editing in chloroplasts, was sent to Rutgers by his mentor, Hans Kossel, before he started his thesis research in Freiburg, Germany. The chloroplast genome is a suitable chassis to accommodate complex operons, such as the genes required for nitrogen fixation, and is likely to be the testing ground for bold projects in synthetic biology.

When did you become a member of ASPP/ASPB

I joined ASPP shortly after arriving in the US in 1982. I was initially lured by the discount on journal subscriptions, publication charges and meeting fees. Soon, I discovered that the annual meetings are a great place to meet people. I look forward to networking at the ASPB meeting every year.

How did the Society impact your career, and what motivated you to become a Founding Member of the Legacy Society?

The American Society of Plant Biologists provides a home for all areas of plant science. When invited, I was ready to join, because I highly value ASPB's educational, outreach and lobbying activities and find it a worthwhile cause to support.

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

Science is constantly changing, and everyone has to chart their own way. On average, I had to change the direction of my research every five years. In other words, I never knew what I would do in five years' time, because in the meantime some major discovery had been made. My advice is that keep an open mind, be part of the network that drives science and you will be successful.

Academic family tree:
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