Joachim W. Messing

1946–2019

BIOGRAPHICAL *Memoirs*

A Biographical Memoir by
Hugo K. Dooner,
Robert M. Goodman,
Pal Maliga,
and Marja Timmermans

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JOACHIM W. **MESSING**

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By Hugo K. Dooner, Robert M. Goodman, Pal Maliga, and Marja Timmermans

Joachim Messing, through a long string of groundbreaking accomplishments in genetics and evolutionary biology, virtually laid the foundations of the multibillion-dollar industries populating the various sectors of the life sciences. His most noteworthy early successes were the development of methods of gene sequencing that greatly simplified this blossoming science as well as the so-called shotgun sequencing of DNA. He sought no ownership protection for his brilliant and original discoveries, enabling research biologists and industrial developers the world over to build on them.

Born in Germany, Messing earned a B.S. in pharmacy in Dusseldorf (1968), an M.S. in pharmacology from the Free University of Berlin (1971), and a doctorate in biochemistry and pharmacy from Ludwig Maximilian University in Munich (1975). After a three-year stint as a research associate at the Max Planck Institute in Munich, Messing moved in 1978 to the University of California at Davis as a research associate in bacteriology. From there he accepted a tenure-track assistant professorship at the University of Minnesota in 1980, rising to full professor in just four years. From 1985 until his unexpected passing in 2019 he was the University Professor of Molecular Biology at Rutgers University, also becoming director of the Waksman Institute in 1988.

Joachim Wilhelm "Jo" (pronounced yo) Messing was born in Duisburg, Germany, on September 10, 1946, the first of the three children of Martha and Heinrich Messing. His mother was a homemaker, and his father, a mason, owned a construction company. Duisburg, a major industrial center during World War II, was severely damaged by Allied bombing. Life was not easy for the family, but Jo was inquisitive and determined, and he became the first in the family to go to college. His youth was also a time in which his love for travel blossomed; he biked through England on his own at 16, and twice traveled around the world, reaching the World's Fair in Osaka in 1970. While earning a B.S. in

pharmacy (1966-1968), he worked at the Deutsche Opera am Rhein, developing this lifelong passion for the opera. He studied pharmacology at the Free University of Berlin from 1968 to 1971, obtaining an M.S. While there he organized the First International Pharmacy Conference in Berlin after convincing Schering AG and independent pharmacies of Germany to provide the funding.

Jo then moved to Munich, where he gained his first research experiences working at the Max Planck Institute for Biochemistry and was mentored as a doctoral student by P. H. Hofschneider and W. L. Staudenbauer.

In addition to marking the beginning his doctoral studies, 1971 was a pivotal year in Jo's personal life as he also met the love of his life, Rita, on a ski trip to Italy. He earned the Ph.D. from Ludwig Maximilian University in 1975, the same year that Rita and he were married. He stayed on as research fellow at the Max Planck Institute until 1978, when he and Rita moved to the University of California at Davis, where Jo had accepted a position as a research associate. That same year, their son Simon was born.

The topic of Jo's dissertation was DNA replication and focused on "minicircular" DNAs of *Escherichia coli*. In a series of four articles appearing between 1972 and 1974 (1-4), the first of which appeared in *Nature New Biology*, he dissected the replication mechanism of these minicircular DNAs. The work was based on previous studies showing that replication of DNA required transcription of a replication origin. Still open was the possibility that protein synthesis was also required. Messing, et al., (1) used treatments with antibiotics known to block protein or, specifically, RNA synthesis, to show that the product of transcription, but not of protein synthesis, was required to initiate DNA replication. The resulting insight into the priming of DNA replication led to Jo's subsequent work on the intermediate products of DNA replication, but more powerfully to development of the tools for shotgun sequencing—a means of assembling long continuous stretches of DNA from randomly cloned DNA fragments based on sequence overlaps.

Jo recounted his attendance at an early lecture where Fred Sanger described the use of chain-termination methods in DNA sequencing. The method required the laborious preparation and isolation of DNA fragments by gel electrophoresis for sequencing. Jo set out to resolve the bottlenecks in the method by making modifications in the single-stranded DNA coliphage M13. He used mutagenesis to introduce restriction-enzyme target sequences, the lac promoter, and the coding region for the alpha peptide of

beta-galactosidase (allowing blue/white screening of plaques carrying inserts) into a nonessential region of M13 (5-7).

This innovation and a torrent of subsequent improvements unleashed the development of cloning as the preferred approach to preparing and processing DNA for sequencing. The series of M13 and pUC ("plasmid University of California") versions created by Jo and his colleagues (8) were widely and freely distributed, and both revolutionized and democratized DNA sequencing at the time. They also led to the invention by others of instruments that automated the process. In collaboration with R. J. Shepherd and other colleagues at Davis, Jo showed the first "proof" of the utility of shotgun sequencing via the M13-based cloning system by sequencing an infectious clone of the double-stranded DNA of cauliflower mosaic virus, a para-retrovirus of plants (9).

In 1980 Jo and family moved from Davis to the University of Minnesota, St. Paul, where he had accepted a tenure-track assistant professorship in biochemistry. There he moved up at stellar speed to full professor in 1984. His commitment to plant sciences is indicated by the fact that, while his appointment was in the College of Biological Sciences, his laboratory was located in the College of Agriculture, together with the laboratories of Burle Gengenbach, Ron Phillips, and Ed Green, who had appointments in the Department of Agronomy. Jo also joined Irwin Rubenstein, Phillips, Gengenbach, and Green to form a group interested in developing applications of genetic engineering to crops.

At Minnesota Jo continued developing molecular biology tools critical to the new recombinant-DNA era. His group generated several sets of improved M13-based cloning vectors. One of the important advances enabled the selective packaging of either strand of a DNA insert. This circumvented the need for directional cloning and allowed both DNA strands to serve as templates in sequencing or oligonucleotide-directed mutagenesis (10). The most significant advance of that time, however, was undoubtedly the development by Jo's group of the pUC series of vectors. These small, double-stranded plasmids included the "multiple cloning site" and a "blue-white" β-galactosidase-based selectable marker, molecular tricks that had made the cloning of DNA into the M13mp phages so simple. Indeed, fragments inserted into the multiple cloning site of pUC would block the production of functional β-galactosidase. Bacteria containing plasmids with cloned DNA could thus be quickly identified as a white rather than a blue colony.

The pUC vectors had one additional major advantage over other cloning plasmids of that time: their copy number was up to 50-fold higher. For a multiple-cloning site to

work, several restriction sites in the pUC backbone had to be mutated via chemical mutagenesis. Inadvertently, a point mutation was created that affected the pUC origin of replication. Plasmid replication is primed at the origin by a short RNA whose activity is normally tuned by an antisense RNA. The pUC plasmids carry a mutation in the anti-sense RNA that causes the copy number to rise considerably. The pUC vectors, as the first high-copy-number plasmids in E. coli, thus became the forerunner of most modern-day cloning plasmids. The impact these tools had on the science community at the time is unmistakable, and on a par with the significance of RNAi and CRISPR-Cas9 technologies developed more recently. The paper by Yanisch-Perron, Vieira, and Jo (11) became the most cited paper of 1985. Moreover, Jo became the most cited scientist of the 1980s.

Among Jo's colleagues at the University of Minnesota were several prominent maize geneticists. Here were exciting opportunities. Jo's "genomics" expertise perfectly complemented his colleagues' extensive knowledge of maize genetics. So, not long after his arrival at Minnesota, Jo directed much of his new lab's efforts into understanding gene structure and function in maize. He joined the Maize Genetics Cooperation and began to attend the annual Maize Genetics meetings.

Here, with the advent of molecular biology, groups were presenting their efforts to isolate the transposable controlling elements Activator (Ac) and Dissociation (Ds). The elegant studies of Barbara McClintock in the late 1940s and early 1950s on Ac and Ds had provided the first evidence that genetic elements transpose and give rise to unstable mutations affecting gene function. Around 1983, the year that McClintock received the Nobel Prize in Physiology or Medicine for this work, several groups reported the cloning of both the Ac and Ds elements. Jo's group then used their earlier shotgun sequencing strategy to determine the nucleotide sequence of the Ac element isolated from Ac wx-m9 (12).

This revealed a structural organization not unlike that of the Tn3 transposon from bacteria, with two major open reading frames (ORFs) diverging from a short intergenic region. The largest ORF was predicted to encode the transposase function of the element. Indeed, Ds elements were found to carry differently sized deletions that disrupt this ORF. All Ds elements, however, shared an 11-base-pair terminal inverted repeat that also defined the ends of Ac. The sequencing of the Ac and Ds elements thus provided a molecular perspective to explain the extensive genetic evidence that, although both Ac and Ds are able to transpose, only the Ac element can drive transposition. Ds elements

seemed to constitute a group of defective elements, arisen via mutations of various kinds from Ac, that are stable unless a functional Ac element is present. This insight into the Ac-Ds transposon system was later exploited for gene tagging in a range of heterologous plant systems.

While at Minnesota, Jo also collaborated closely with Rubinstein and Phillips in their research focused on zeins, the major storage proteins in the endosperm of maize. Due to their economic importance, and likely also their abundance, zeins were of broad interest. However, although primarily 19- and 22-kDa in size, the zein protein fraction proved to be quite heterogeneous, which complicated sequencing efforts and other biochemical analyses. The realization, by Jo, Rubinstein, and Phillips, that zeins are synthesized on polysomes bound to the surface of protein bodies was therefore pivotal, as it provided a means to isolate individual zein transcripts. Indeed, in the late 1970s the first zein mRNAs were isolated from polysomes present in protein-body preparations. In the few years following, Jo's group, often in collaboration with Rubenstein's lab, published a number of papers on sequence analyses of zein genes, resolving the complexity of the 19- and 22-kDa zeins by demonstrating that these represent structural variants within a single large multi-gene family (13, 14).

In 1985 Jo moved to Rutgers University to assume the research directorship of the Waksman Institute while continuing his own research on the zein genes. In 1988 he became director of the Institute. Upon arriving at Rutgers, Jo took on the reorganization of the Institute by bringing in scientists who shared his vision of scientific excellence. His first recruit was Daniel Klessig, whom he had allegedly met in a hot tub at a meeting in Japan. Klessig was appointed associate director and made important contributions to building up the Waksman Institute during his stay at Rutgers (1985-2000). The second recruit was Richard Ebright, hired straight out of grad school at Harvard University. Not much later, Jo hired one of us (PM), after we had met at the Plant Course in the Cold Spring Harbor Laboratory. Jo was co-instructor of that course for five summers, between 1984 and 1988. He loved the scientific atmosphere at Cold Spring Harbor, where he spent the summers with his family. Sometimes he would disappear

Nobel laureates meeting 1969 in Lindau, from left: W. Löwe, Mrs. S. Waksman, Selman A. Waksman and Jo Messing.

and on those occasions he most likely would be engaged in discussions with Barbara McClintock about the transposable elements of maize or some other genetic problem.

With advances in random cDNA cloning strategies, it became possible to focus attention on the less abundant zeins. The 10-kDa zein protein, in particular, caught Jo's attention. It held promise for improving the nutritional value of maize seed, which generally is deficient in essential amino acids such as lysine, tryptophan, and methionine. The inbred line BSSS-53 was characterized by a seed-methionine content 30 percent higher than that of the most commonly studied maize varieties, and this trait was linked to increased expression of the 10-kDa zein. Based on a partial amino acid sequence, which revealed a greater than 20 percent methionine content, it was possible to isolate a cDNA for Zps10, the single 10-kDa zein gene, via hybridization of a degenerate oligonucleotide probe (15).

Having the gene in hand, Jo and his group shifted their attention to understanding its regulation during endosperm development. They showed that enhanced Zps10 expression in BSSS-53 depended on the activity of an unlinked regulatory locus, dzr1 (aka Zpr10), which controls accumulation of Zps10 mRNA and, thereby, protein, at the post-transcriptional level (16). Interestingly, while the dzr1 allele of most inbreds, including that of BSSS-53, are dose-responsive in function, the Mo17 allele was shown to be imprinted and to condition a dominant low 10-kDa zein accumulation phenotype only when transmitted through the female (17).

The cloning of dzr1, which was necessary for dissecting the post-transcriptional regulatory mechanism and its allele-specific imprinting, proved difficult, however. Transposon tagging was widely used to clone genes of interest, but this approach required a readily visible phenotype, which wasn't the case here. Encouraged by the releases of high-density genetic maps and yeast artificial chromosome (YAC) libraries for maize, Jo's group decided to try a map-based cloning approach. Note, this was the early 1990s, and many years ahead of even a draft maize-genome sequence. Nonetheless, dzr1 was shown to localize to the 22-kDa zein gene cluster on chromosome 4S (18). Thus, perhaps bringing the work full circle, steady state Zps10 mRNA and protein levels are regulated at protein bodies. The basis for the epigenetic regulation of the Mo17 dzr1 allele remains unresolved, but benefits of the high methionine trait for seed quality could be demonstrated in several feeding trials of 2-day-old chicks.

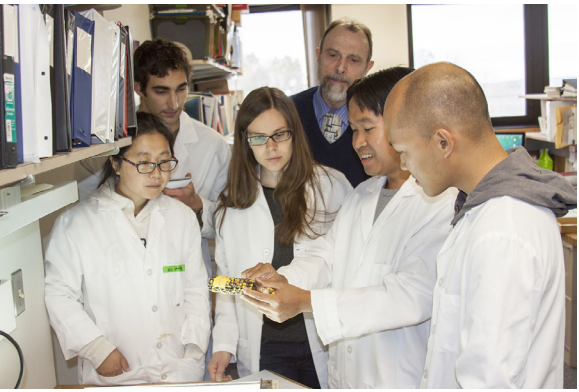
In the late 1990s, when it became possible to isolate DNA inserts larger than 100 kb in bacterial artificial chromosomes (BACs), Jo's interest turned to the chromosomal organi-

zation of the 19- and 22-kDa zein genes that his lab at Minnesota had sequenced earlier. The analysis of these BACs revealed that the zein genes were organized in families arrayed in tandem, but that only some of them were expressed (19, 20). Jo was always interested in the latest technologies that might aid in answering biological questions and often adopted them in his research. In addition to BAC cloning and next-gen sequencing, he set up a transformation pipeline in his lab and showed that an RNA-interference construct derived from a single 22-kDa zein gene could knock out expression of the entire family, producing a dominant opaque seed phenotype, similar to that produced by mutations in the Opaque2 transcriptional activator (21).

The availability of large DNA insert libraries in maize and its close relative, sorghum, allowed for an evolutionary comparison between these two grass species, so Jo and Jeff Bennetzen entered into a major collaboration to analyze the comparative structure of a set of selected genomic regions. This collaboration led to the publication of several important papers that detailed the evolution of genomic regions chosen because they were genetically well defined in maize (22, 23). A major outcome of this fruitful collaborative study was the determination of the evolutionary split between the progenitors of the maize and sorghum genomes (24).

Jo's love affair with DNA sequencing lasted his whole life. After the turn of the century, when next generation sequencers became more widely available and plant genomes began to be sequenced, Jo leapt at the opportunity to contribute to this effort. He set up a large DNA sequencing operation in his lab and became involved in the international project to sequence rice, the second plant and first crop genome to be sequenced (25). Rice is the most widely consumed staple food in the world. Having major syntenic relationships with the other cereal species, rice served as a model plant for the grasses, and its map-based sequence proved useful in the identification of several genes underlying agronomic traits.

Jo's lab was also involved in the subsequent sequencing of other important plant species: Sorghum bicolor (26), the wild-wheat relative Brachypodium (27), the aquatic plant Spirodela (28), and the African cereal teff (29). Each of these was significant in its own way. In addition to being a food crop, sorghum is grown around the world for fodder, fiber, and fuel. The genome sequences of the closely related sorghum and maize, published in the same year, provided investigators with the opportunity to compare these genomes, which contain much more repetitive DNA in the form of LTR (long terminal repeat) retrotransposons than the first two plant genomes sequenced, rice and Arabidopsis.



Messing lab 2014, from left, Wei Zhang, Paul Fourounjian, Anna Zdepski, Jo Messing, Nelson García, and José Planta.

Brachypodium dystachium was chosen for sequencing because of its much smaller genome compared with wheat, rye, and other cool-season cereals. Its sequencing allowed, for the first time, whole-genome comparisons between members of the three most economically important grass families, represented by rice, sorghum-maize, and Brachypodium. One of the surprising outcomes from these comparisons was that the five-chromosome karyotype of Brachypodium could be explained by nested insertions of entire chromosomes into centromeric regions.

The cereal teff is the staple grain crop in the horn of Africa, preferred over other cereals because of its nutritional profile, low input demand, and tolerance of marginal farmland. Teff is an allotetraploid that arose ~1.2 million years ago, yet, surprisingly, has undergone none of the major rearrangements or biased gene loss seen in other allopolyploids. It is clear from the above that Jo enjoyed working in teams: all of the genome projects were multi-investigator efforts, just as the shotgun sequencing of the cauliflower mosaic virus genome had been in the early 1980s.

One of Jo's scholarly activities at Minnesota had a significant impact on his future life. During the summers of 1980-83 he set up an M13 cloning and sequencing course in the department. One of the instructors was Daniel Vapnek, a genetics professor at the University of Georgia who later became the chief scientist of Amgen, a highly successful biotech company. When Vapnek retired, he was looking for investments in biotech startups. Through his acquaintance with Jo, he became the initial investor in BioArray Solutions, a company that he had set up at Rutgers in 1999 with Michael Seul. Jo served as chair of the Scientific Advisory Board of BioArray Solutions till 2008, when the company was acquired by Immunocore. The company's profile was DNA-based diagnostic tests, to which Jo actively contributed, as witnessed by his inventorship on several patent applications assigned to BioArray Solutions.

In a letter to Rita Messing upon Jo's passing, Vapnek recognized the importance of Jo's freely available engineering tools to the success of Amgen. In recognition of Jo's contributions to science, Vapnek and Seul endowed the Selman A. Waksman Chair in Molecular Genetics at Rutgers, of which Jo was the first recipient in 2009. Jo was also a catalyst in the creation of a second endowed chair, the Joachim Messing Endowed Chair in Molecular Genetics, in 2016. Jo initiated the process by offering the money that came with the Wolf Price in Agriculture toward the creation of this second endowed chair in the Waksman Institute.

Jo Messing in 2019.

In hindsight, Jo's refusal to take patents on the core biotechnology tools was more of an exception than a rule. His many dealings with industry (he served on the board of eight biotech companies) and his service as expert witness at court trials on behalf of biotech companies make it clear that he fully understood the value of intellectual property. While at Rutgers he also filed for patent protection on discoveries made in his laboratory. He is the principal inventor on five U.S. patents and eight patent applications on projects ranging from the use of small RNAs to improve crop productivity in maize and duckweed to improvement of the nutritional quality of crops.

This picture of Jo would not be complete without mentioning the role of his wife, Rita, in the operations of the Waksman Institute. The Messings made an all-out effort to make investigators feel part of the "Waksman family" by organizing social functions, such as Institute-wide picnics. Every summer, they had a pool party where they invited all of the Messing lab members and their families. Jo loved opera, and the Messings often invited job candidates to the Metropolitan Opera to highlight the attractiveness of the area. Rita also organized the International Women's Group at Rutgers, a voluntary organization where wives of international professors and students meet regularly to share information about their home country, attend cultural events as a group, cook and share ethnic dishes, and exchange practical information about the society around them.

In recognition of his scientific accomplishments, Jo was elected to the U.S. National Academy of Sciences in 2015 and received many other awards and honors. Among them, he was elected Fellow of the American Association for the Advancement of Sciences in 2002, named a member of the German Academy of Sciences Leopoldina in 2007,

awarded the Wolf Prize in Agriculture in 2013, and elected Fellow of the American Academy of Microbiology on 2015 and of the American Academy of Arts and Sciences in 2016.

Most anyone who had lunch or dinner with Jo would have heard him explain that he wanted his invention of the M13 cloning and shotgun sequencing to be freely available. He declined to file for patent protection so that people would be able freely to use his invention to explore new applications. These license-free biotechnology tools are one of his legacies. His vision was fulfilled, because most companies today use his pUC vector derivatives. Jo's contributions enabled genome sequencing from viruses to tumors, from crop plants to humans. Industries have been built on the foundations that he laid; in the life sciences, every company from seeds to pharmaceuticals, every university research laboratory, and the work of thousands of entrepreneurs attracting billions in investment have benefitted from the inventive mind and the dogged work ethic of Jo Messing.

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