Cell

Walter Fiers (1931–2019)

Walter Fiers, professor in Molecular Biology, passed away on July 28, 2019. From the very beginning of his scientific career, Walter Fiers has made groundbreaking contributions in a vast number of domains ranging from pure molecular biology to molecular virology to molecular immunology and biotechnology. His work revealed the existence of circular genetic elements, helped to resolve the universal genetic code, included the first-ever deciphered full-genome sequence, and contributed to the discovery of mRNA splicing in eukaryotes. Moreover, he pioneered molecular cloning, recombinant expression, and the study of interferons and several cytokines. He also elucidated how influenza A viruses manage to evade the host adaptive immune system and how these viruses can acquire the capacity to spark a pandemic outbreak.

Walter Fiers was born on January 31, 1931 in the Westhoek in Ypres, Belgium, where he grew up in a city built on the ruins of the First World War. In 1954, he obtained the degree of engineer at the State School of Agriculture—later the State Faculty of Agriculture Sciences—at Ghent University. During the final year of his studies, Walter Fiers met the young and inspiring scientist Laurent Vandendriessche (professor in physiological chemistry, Ghent University) who convinced Walter to start a PhD in his laboratory. In those pioneering times of biochemistry, the lab, tools, and methods had to be built from scratch, and Walter eagerly devoted his doctoral research to the functioning of enzymes.

During his PhD, Walter Fiers quickly realized that knowledge and expertise was not always found in the immediate vicinity. From 1956 to 1957, he learned biochemical and subcellular separation techniques in the world-renowned Carlsberg laboratory in Copenhagen under the direction of Heinz Holter, a good friend of Niels Bohr. Exceptionally, Walter Fiers obtained the diploma of Aggregate for Higher Education in Biochemistry, allowing him to teach at university level, 3 years before his PhD diploma, which he obtained in 1963 under the supervision of Laurent Vandendriessche.

By the time he earned his PhD, Walter Fiers would have already worked for 3 years as a scientist in two outstanding research laboratories in the United States. In the summer of 1960, he and his wife moved to California, where he joined the lab of Robert L. Sinsheimer at the renowned California Institute of Technology (CalTech, Pasadena, CA, USA), supported by a fellowship from the Rockefeller Foundation and the Belgian National Science Foundation (NFWO). In the early 1960s, it was known that the "genome of the minute" *Escherichia coli* bacteriophage PhiX174 consisted of a single-stranded DNA chain of approximately 5,500 nucleotides. Although phage genetics was in vogue because it allowed mapping of genes on a DNA genome, still very little was known about the physicochemical structure of DNA molecules. By using a series of exo- and endonucleases combined with analytical velocity sedimentation experiments, Walter demonstrated that the genome of phage PhiX174 is a circular molecule. This conclusion represented a breakthrough finding, and the experimental evidence that Walter had gathered to prove this point was published in three research articles, all submitted on the same day, that appeared in the *Journal of Molecular Biology* in 1962 (Fiers and Sinsheimer, J. Mol. Biol. *5*, 408–419; Fiers and Sinsheimer, J. Mol. Biol. *5*, 420–423; Fiers and Sinsheimer, J. Mol. Biol. *5*, 424–434). In the fall of 1962, Walter moved to Madison to join the laboratory of Har Gobind Khorana at the University of Wisconsin (USA). Khorana would later go on to receive the Nobel Prize in Medicine and Physiology, together with Robert W. Holley and Marshall W. Nirenberg, for their contribution to the elucidation of the genetic code. In the lab of Gobind Khorana, Walter Fiers purified and characterized an exonuclease from *Lactobacillus acidophilus*. Gobind Khorana had developed a method to synthesize oligodexoynucleotides, which was far from obvious at that time, and it was decided at some point to feed the exonuclease Walter was characterizing with different types of these polynucleotides. It turned out that the enzyme was picky and could not hydrolyze polyadenylic or polyuridylic acid, whereas thymidine oligonucleotides were readily turned into single nucleotides by the enzyme. In one of the papers reporting this work, the authors discussed the possibility of using the phosphodiesterase for the sequence determination of polynucleotides (Fiers and Khorana, J. Biol. Chem. *238*, 2789–2796).

Walter Fiers

Using nucleases to determine the sequence of a polynucleotide is exactly what Walter Fiers had in mind after his return to Belgium in 1963. His aim, however, was extremely ambitious: to determine the sequence of the complete genome of a bacteriophage. According to many of his peers, this was an impossible project, and it was difficult to raise the necessary funding to start it. However, the seeds were laid to develop the new scientific discipline of molecular biology at Ghent University, and in 1967, Walter Fiers became director of the newly established Laboratory for Molecular Biology.

His lab was next to that of Marc Van Montagu and Jozef Schell in the Ledeganckstraat in Ghent, which created an inspiring and enthusiastic scientific atmosphere. Notably, in the 1980s, Jozef Schell, Marc Van Montagu, and colleagues would go on to identify the natural mechanism of gene transfer from *Agrobacterium* to plants, resulting in plant molecular genetics tools that revolutionized fundamental and applied knowledge in plant biotechnology. Walter recruited very talented PhD students, and his team set out to determine the genome

Marc Van Montagu, Walter Fiers, and Jeff Schell at the workshop on "Restriction enzymes as tools in molecular biology" (1974) in the Abbey of Drongen, close to Ghent in Belgium. The reputation of the Ghent teams attracted many famous molecular biologists to this meeting, such as Walter Gilbert, Fred Sanger, Werner Arber, Daniel Nathans, Howard Goodman, Walter Doerfler, Richard Roberts, Herbert Boyer, Heinz Schaller, Sherman Weissman, Ray Wu, and many others.

sequence of the *Escherichia coli* bacteriophage MS2. This phage was chosen because it provided a means to isolate high amounts of intact RNA, as it is sheltered by the phage coat, and because the MS2 genome serves as a mRNA for this virus. The techniques that were available at that time did not compare to modern sequencing techniques and presented an extreme challenge. The experimental approach was to chop the radiolabeled RNA genome of MS2 into pieces with RNase T1 or pancreatic RNase and then isolate the resulting oligonucleotides. Two-dimensional gel electrophoresis, paper chromatography, and treatment with exonucleases were subsequently used to deduce the primary nucleotide sequence of these oligonucleotides. Needless to say, the project required a long-term vision and perseverance. A typical PhD thesis in those days in the Fiers' lab described the sequence of 5 to 10 pentanucleotides of phage MS2. Over time, overlapping sequences were spotted, which slowly, piece by piece, allowed reconstituting the genome sequence of MS2. In 1972, Min Jou et al. published the complete nucleotide sequence of the MS2 coat protein gene (Min Jou et al., Nature *237*, 82–88). This was the first time that the complete sequence of any gene coding for a protein was determined. Moreover, the work allowed confident annotation of codon usage, because the peptide sequence of the MS2 coat protein had just been determined by Joel Vandekerckhove and Marc Van Montagu (Vandekerckhove et al., Arch. Int. Physiol. Biochim. *77*, 175–176). In 1976, the *Nature* paper "Complete nucleotide sequence of bacteriophage MS2 RNA: primary and secondary structure of the replicase gene" was truly a landmark achievement: it was the first-ever published complete genome (Fiers et al., Nature *260*, 500–507). Shortly after, Fred Sanger reported the first sequence of a DNA virus genome (Sanger et al., Nature *265*, 687–695), the PhiX-174 virus, which Walter Fiers had studied earlier in his career.

In the early 1970s, Walter Fiers had established mammalian cell culture in his laboratory. So, it was possible to culture a mammalian virus and, of course, to sequence the genome of such a virus. One such virus was SV40, which was considered very interesting because it had the potential to cause tumors in inoculated animals. Unravelling the SV40 genome sequence could thus shed light on the molecular basis of tumor formation. Walter Fiers published the complete nucleotide sequence of this eukaryotic DNA virus in 1978 (Fiers et al., Nature *273*, 113–120). In addition, the SV40 sequencing work in Ghent contributed to the discovery of RNA splicing in eukaryotes (Haegeman and Fiers, Nature *273,*70–73). Today, many plasmids used for expression of proteins in eukaryotic cells still contain genetic elements of the SV40 genome (e.g., the SV40 origin of replication, promoters, polyadenylation signals). The fundamental molecular biology research of the SV40 virus was the basis for Walter Fiers to initiate a cancer research line in his lab.

In a subsequent period, research focused on the identification of the genes that code for interferons and cytokines. Interferons initially aroused attention due to their ability to restrict viral infections, but they were also thought to be able to combat cancers of viral origin. Cytokines are proteins that play an important role in the regulation of our immune system (e.g., interleukin-2 is a growth factor for T cells). The next milestones in the research career of Walter Fiers's team were the cloning of the genes for and the recombinant production of interferon beta (fibroblast interferon), interferon gamma (immune interferon), interleukin-2, tumor necrosis factor (TNF), and interleukin-6. Being an engineer by training, Walter Fiers quickly realized the practical and medical implications of trying to produce interferon beta, as well as other cytokines with potential medical applications in heterologous expression systems. This would, in principle, make it possible to produce large quantities of these proteins and, if desired, to make mutants with altered properties. Together with Erik Remaut, who had joined the Fiers laboratory in 1966, a prokaryotic expression system based on the powerful leftward promoter of phage lambda was developed. This allowed the team to prove that *Escherichia coli* could indeed produce biologically active human interferon beta in a practical, inducible way (Derynck et al., Nature *285*, 542–547; Derynck et al., Nature *287*, 193–197). At that time, several companies producing recombinant insulin also used this expression system. His worldwide reputation in gene cloning and recombinant protein expression resulted in him being made a member of the Scientific Council of Biogen company (founded in 1978) together with colleagues such as Walter Gilbert, Charles Weismann, Phillip Sharp, Heinz Shaller, and Kenneth Murray.

The zest of his laboratory for nucleotide sequencing remained high, and in the late 1970s, Walter Fiers set out to determine the primary structure of the major antigenic spike protein of influenza A viruses, which cause the flu. It was known that influenza A viruses can swiftly escape from vaccine-induced or naturally acquired immunity by a process known as antigenic drift. This means that the antigenicity of the viral hemagglutinin and neuraminidase, the two main spike proteins of influenza viruses, changes over time. As a result, people can get the flu many times in life. However, how this antigenic drift was controlled genetically was unclear at the time. Moreover, influenza A viruses were known to occasionally cause pandemics, a fearful event during which an influenza virus with an antigenically highly different hemagglutinin emerges and rapidly spreads in the human population (notorious examples are the 1918 Spanish flu and the Hong Kong flu of 1968). In 1980–1981, the Fiers lab not only reported the complete sequence of the

hemagglutinin gene of the influenza virus that caused the Hong Kong flu pandemic of 1968, but also elucidated the mechanism of antigenic drift and showed that the hemagglutinin in the human Hong Kong flu virus was acquired from an avian influenza virus (Min Jou et al., Cell *19*, 683–696; Verhoeyen et al., Nature *286*, 771–776; Fang et al., Cell *25*, 315–323). Later work showed that one part of the virus that is accessible on the surface is remarkably conserved. This conserved part is the extracellular domain of the matrix 2 membrane protein—in short, M2e. Could this conserved domain, which was seemingly difficult for the virus to change, be targeted by a recombinantly produced vaccine? Walter Fiers and collaborators showed that an experimental vaccine against M2e protected mice against influenza A virus infection (Neirynck et al., Nat. Med. *5*, 1157–1163). In collaboration with a pharmaceutical company, this M2e-based vaccine was later successfully tested in a phase I clinical trial: the vaccine was safe and induced M2e-specific antibodies in the volunteers. Today, many research laboratories and biotech companies are still investigating ways to implement M2e as an important component for a universal influenza vaccine.

In a third period of his career, his main focus was on the development of one such universal influenza vaccine, as well as the biology and mechanism of action of TNF. TNF initially created much hope as an anti-cancer cytokine that could shrink certain tumors in a spectacular way. But TNF has a Janus face—it is also a cytokine that plays a crucial role in numerous inflammatory conditions such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease. TNF-blocking medicines are currently the best-selling drugs in the world, with a market value of $20 billion a year. Research in Walter Fiers' team on how TNF kills cells has, among other things, identified TNF-induced necrosis, a completely new type of cell death, which was later characterized by other groups as necroptosis, with promising therapeutic applications that have currently entered phase II clinical studies by GlaxoSmithKline.

The high quality and impact of the scientific output of Walter Fiers is impressive (706 articles, 52,000 citations, and an h-index of 114), but the constant focus on a number of major topics in molecular biology is especially astonishing. Together with Marc Van Montagu and Jozef Schell, Walter Fiers turned Ghent University into an important hub for molecular biotechnology. Furthermore, Walter was one of the founding fathers of the Flemish Institute of Biotechnology (VIB), an institute successfully combining basic research in biomedical and plant biotechnology at different Flemish universities with a common tech transfer program. Walter received numerous awards for his research, such as the Francqui Award (1976), the Dr. Beijerinck Gold Medal for Virology (1986), the Artois-Baillet Latour Prize (1989), the Carlos J. Finlay Prize (1989), and the Robert Koch Prize (1991). In 1990, Walter Fiers was awarded the title of baron. He also belongs to the select group of the 100 most-cited authors in the biotech sector. Walter Fiers retired in 1997 and became a freelance employee at Ghent University, where he continued to focus on research into the universal flu vaccine.

If a phylogenetic analysis were made of the scientific descendants of Walter Fiers, it would be clear that his lab has been a breeding school for many successful national, international, academic, and industry researchers. The impact of his research findings cannot be overestimated. Everyone who has worked with him was impressed by his unique combination of a broad ambitious vision, coupled with a remarkable sense of detail and a technology-focused approach. Walter Fiers was a modest man, and at conferences he carried out intense discussions, both with his peers and with early-stage scientists, driven by his curiosity to get new insights that could help to solve a scientific problem. Walter Fiers encouraged us to give the best of ourselves, as he did himself, and to think carefully before embarking on a sidetrack of a project. If he wanted to understand something, he would keep asking in a Socratic way until we were tongue tied—of course, we tried to avoid that as much as possible. Although his prime passion was science, Walter Fiers also genuinely cared about the personal well-being of his collaborators and their families and kept doing so even long after his retirement.

Today, we mourn for Walter Fiers with a great sense of respect and gratitude. We had the pleasure to enjoy his exceptional scientific career that has ultimately inspired hundreds of scientists for one of the most beautiful human activities alongside the arts, namely the unlimited passion for basic scientific research with an eye for its possible applications. This breadth in thinking from gene to clinic was one of the characteristics of Walter Fiers. We are very grateful to have experienced such a great man of science and a kind, very motivating mentor.

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