

## Profile of Eckard Wimmer

The finding caused an uproar. Researchers at Stony Brook University in New York had engineered poliovirus in a test tube (1). The discovery, led by Eckard Wimmer, elected in 2012 to the National Academy of Sciences, dispelled the belief that viruses require a live host to grow and spread.

The thought of synthetic viruses terrified an American public still reeling from 9/11 and the subsequent anthrax attacks. What if the technology to engineer viruses wound up in the hands of bioterrorists? However, today, just a decade later, it is widely accepted that the ability to engineer viruses also allows researchers to develop viruses that work as synthetic vaccines, to carry genetic material into a cell for use in gene therapies, or to preferentially attack cancer cells (2).

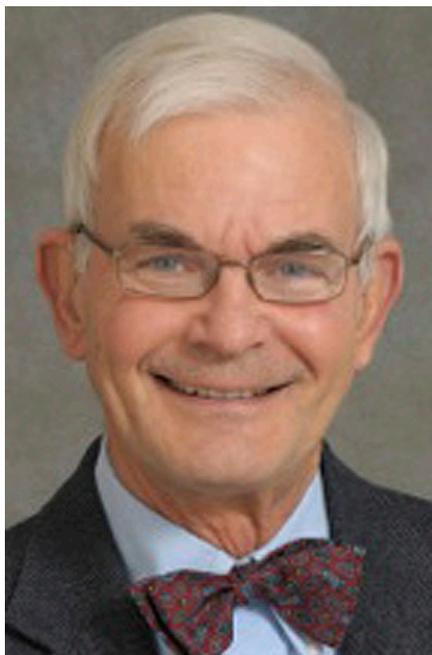
Wimmer says his motivations for engineering polio were strictly scientific. Wimmer had solved the code—or genome sequence—for polio in 1981, but his goal, he says, was “to boot the synthesized genome for it to become a virus.” The boot worked.

Few realized that Wimmer had done something extraordinary for science and society. Viruses, Wimmer and others have long believed, hold a unique place in biology: Outside the body, they behave like chemicals, but, once inside, they propagate in true Darwinian fashion. “Viruses are chemicals with a life cycle,” Wimmer says. “Once they get into living cells, they begin to replicate, following the laws of heredity and genetic variation.” Put another way, viruses hover on the boundary between life and nonlife.

Wimmer’s Inaugural Article brings him one step closer to pinning down the inner workings of poliovirus (3). In this case, Song et al. looked for the parts of the polio genome that make it so deadly—that is, the parts of the code that allow the virus to replicate and kill host cells. The researchers found two hidden signals: nucleotide sequences dubbed  $\alpha$  and  $\beta$ .

### War Child

Wimmer’s interest in science coincided with growing doubts about his Protestant upbringing. Born in Berlin in 1936, Wimmer survived tragedy and war early in life. His father died when Wimmer was just three years old. By then, Hitler had begun his march across Europe. Wimmer’s mother whisked him and his two older brothers to her parents’ place in Saxony, where the family largely escaped the violence. In Saxony, however, Wimmer’s grandfather was labeled a capitalist, a term that was anathema to the com-



Eckard Wimmer.

unist regime that had taken root in East Germany. The family fled again, ultimately becoming refugees in what was then West Germany. Through so much dislocation, Wimmer says, “the family lost its roots.”

Wimmer suffered nightmares throughout his teen years and long thereafter. Forced to question the senselessness of human strife early on, Wimmer emerged from his childhood an avowed atheist. “Every child who has been exposed to warfare or lives in an area with warfare carries a burden. . . for his whole life,” Wimmer says.

The desire to understand life via science, not some higher power, would ultimately become integral to Wimmer’s later career. Wimmer chose to study chemistry in large part because his father studied chemistry. After finishing his undergraduate degree in chemistry at the University of Rostock in Germany in 1956, Wimmer went to the University of Göttingen in Germany for graduate school. There he began his earliest work in structural analysis and synthesis of natural compounds. His task was finding the structure of rhodomycinons (4), a class of compounds whose derivatives have antitumor activities.

During his postdoctoral work in the early 1960s, also at Göttingen, Wimmer enrolled in a course in microbiology. There he heard about biochemistry, then a fledgling field in Germany. Biochemistry arose out of the ashes of

vitalism—the belief that chemicals in living systems are somehow distinct from the chemicals in inorganic systems, such as salt and rocks. That notion was shattered in 1828, when German chemist Friedrich Wöhler synthesized the organic compound urea from inorganic precursors (5).

By the 1940s, theoretical physicists and biochemists had begun to wonder about what constitutes life. Wimmer was enthralled. Biochemistry squared perfectly with his worldview, the idea that something as seemingly profound as life could be pared down to its simplest—chemical—form.

While completing his second postdoctoral fellowship at the University of British Columbia in Vancouver in the mid-1960s, Wimmer attended a talk on viruses and had his eureka moment. “It was clear, even though it wasn’t mentioned in that talk, that if viruses can be crystallized just like chemicals, it must be possible to describe their components, the virions, with an empirical formula,” he says (6). At that conference, Wimmer discussed the idea with Elias Reichmann, a plant virologist at the University of Illinois in Urbana–Champaign. Reichmann asked Wimmer to join his laboratory. “Now I was stuck and had to work on viruses,” Wimmer jokes.

### Adopting Polio

At Reichmann’s laboratory, Wimmer initially worked with plant viruses, his mentor’s specialty (7–9). However, that topic of study proved time-consuming, as both plants and their viruses are generally slow-growing. A colleague suggested that Wimmer take a look at poliovirus, which was fast-growing. “Poliovirus fulfilled all my expectations,” Wimmer says. “You could grow it in six hours.”

In 1968, Wimmer became an assistant professor in the microbiology department at Saint Louis University in Missouri. There, Wimmer found himself in an empty laboratory with almost no funding. And yet, he recalls, “I was professionally independent for the first time in my life and in control of my career. I felt like a king.” His first grant application to sequence the polio genome was roundly rejected, as no one thought it could be done. Then, in 1969, Wimmer was invited to spend six months working with David Baltimore, a biologist then at the Massachusetts Institute of Technology in Cambridge, MA. Baltimore taught Wimmer the ins and outs

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member’s Inaugural Article on page 14301 in issue 36 of volume 109.

of polio research (10). Eventually, Wimmer was able to achieve the goals postulated in his original grant application.

In the mid-1970s, Wimmer moved to New York to help start the microbiology department at the then-new state school at Stony Brook. He has remained there ever since. Soon, Wimmer and his colleagues deciphered poliovirus' chemical structure, a finding that provided the foundation for Wimmer's subsequent efforts to map the biological information hidden in the viral genome, a feat he accomplished in the early 1980s. The resulting gene map proved for the first time that the poliovirus genome encodes a single giant protein, the polyprotein (11–13). Although the presence of such a polyprotein had been predicted, Wimmer's work proved its existence (14). He was also able to identify the parts of the genome that created the enzymes needed to cleave the polyprotein (15).

### Deconstructing Viruses

The genome sequence gave Wimmer a sweeping view of poliovirus. However, he didn't yet know the intricacies of its workings. That picture began to grow clearer in the late 1980s. In 1988, Wimmer found that some viruses trigger protein synthesis in a manner that deviated entirely from the established mechanism, known as "cap-dependent translation." Using a virus related to poliovirus, Wimmer discovered that an unusually large segment of the viral genome (450 nt) was attracting ribosomes, the protein synthesis factories of cells. The attraction enabled the ribosomes to initiate protein synthesis. Wimmer called this segment an internal ribosomal entry site (IRES) (16, 17). Interestingly, Nahum Sonenberg, a biochemist at McGill University in Montreal, made the same discovery at about the same time (18).

Today, that finding may hold the key to treating glioma, a deadly brain cancer. IRES elements differ from virus to virus, yet they can be exchanged between viruses through genetic engineering. Wimmer's laboratory had been creating these chimeras to see how IRES elements work. Matthias Gromeier, who was then a postdoctoral fellow in Wimmer's laboratory, replaced the IRES from polio with the IRES from human rhinovirus. To his surprise, he found that the resulting chimera grew well in glioma cells but not in healthy cells of the central nervous system (19). A modified form of the chimera is currently in clinical trials for the treatment of glioma (20).

Following his discovery of IRES elements, Wimmer began investigating just how poliovirus enters the cell. Animal viruses, like poliovirus, enter organisms by binding to a specific receptor on the sur-

face of a cell. Once inside the cell, the virus releases its large genome, programmed to make more of itself. Wimmer wanted to find the cellular receptor that polio subverts, so he inserted human genes into mouse cells to make them susceptible to polio (mice lack a polio receptor and are thus not susceptible to the disease). Then, using an antibody, he combed through millions of mouse cells to find the receptor it bound to. By cloning those rare mouse cells, Wimmer identified the receptor, which now goes by the name CD155 (21). Subsequent studies revealed that tumor cells overproduce this receptor, revealing it to be a good biomarker for cancer (22).

### Launching Life

It had been known for decades that inserting a purified, or naked, form of the RNA genome of poliovirus into cells could prompt it to complete its life cycle and produce more virus. However, it took until the 1990s for Wimmer to realize that he just might be able to create poliovirus without cells—or without a live host. In 1991, his team mashed human cells and stripped them of essential elements, including nuclei and mitochondria. He was left, he says, with a very clear juice. Then, Wimmer mixed the polio genome into the juice and tested whether it would produce more of itself in this new medium. Could the polio, in other words, be "booted to life"? When the boot worked, it "simplified the study of how viruses replicate," Wimmer says. "It showed that we don't really need nuclei and this and that" (23).

It would take another 10 years for Wimmer to show that real poliovirus is not required for replication to occur. By returning to the chemical structure of the polio genome he deciphered in 1981, Wimmer and his collaborators began making the entirety of the virus from scratch. That required stringing together polio's 7,500 nt, a daunting task (1).

Nobody had ever built a virus before. The Defense Advanced Research Project Agency initially provided approximately \$200,000 for the project, a relatively small amount, later expanded by approximately the same sum. According to Wimmer, the group barely managed to limp over the finish line when they published in 2002.

That work triggered another landmark finding. In 2002, Wimmer's team inserted 28 nt changes, or "watermarks," into the synthetic genome to demonstrate that the virus growing from it was not a laboratory contaminant. Surprisingly, one of those changes weakened the virulence of the synthetic virus. That observation has now become the basis for constructing a virus that preferentially targets cells in neuroblastoma, a rare childhood cancer (24).

Although many scientists hailed Wimmer's achievement in creating po-

liovirus in a test tube, he also faced intense criticism, even ridicule. However, the technology to invent viruses already existed, Wimmer says. All he did was put it to the test. Several years later, Wimmer published an essay in the journal *EMBO Reports* reflecting on his 2002 publication and the subsequent uproar (25). In it, he acknowledges that creating viruses from scratch does, in theory, negate our ability to eradicate those viruses. He also notes that any eradication plan must take into account this reality. That burden is the consequence of progress, he says, of which the synthesis of poliovirus is a part. Case in point: Polio has proven so resistant to eradication that the World Health Organization and other private research foundations are now funding efforts to develop new polio vaccines (26). Synthesizing poliovirus, Wimmer continues, fits under the National Institutes of Health "dual use research of concern" designation—that is, situations in which the same technology can be used for malfeasance or human betterment (27).

The ability to create viruses from scratch provides amazing power to treat and even solve some of today's most pressing medical concerns, Wimmer says. For instance, after Wimmer engineered polio, another research team resurrected the long-lost flu virus of 1918 (H1N1), a deadly pandemic that killed between 50 and 130 million people. The resurrection let the team decipher what made the flu so lethal (28). Their work is now yielding insights into its modern-day cousin, the bird flu (H5N1).

### Still on the Clock

In the nearly four decades that Wimmer has spent at Stony Brook, he has earned a number of awards, including the Beijerinck Virology Prize from the Royal Netherlands Academy of Arts and Sciences in 2010 and the Robert Koch Gold Medal in 2012 for his lifelong research on viruses. At age 76, Wimmer could easily retire, spending more time with his wife, Astrid, whom he met in Germany; and his two children and three young grandchildren. And he would be able to focus on his hobbies, which include singing, gardening, and skiing.

However, Wimmer feels retirement would mean leaving behind too much and says he can't bear to part with his polio research—not when the promise of synthetic vaccines is so close at hand. The hope is that synthetic vaccines will one day cost less and cause fewer side effects than conventional vaccines (29, 30). Wimmer says he hopes to "see these [synthetic] vaccines developed in my lifetime."

Sujata Gupta, *Freelance Science Writer*

- Cello J, Paul AV, Wimmer E (2002) Chemical synthesis of poliovirus cDNA: Generation of infectious virus in the absence of natural template. *Science* 297(5583):1016–1018.
- Wimmer E, Paul AV (2011) Synthetic poliovirus and other designer viruses: What have we learned from them? *Annu Rev Microbiol* 65:583–609.
- Song Y, et al. (2012) Identification of two functionally redundant RNA elements in the coding sequence of poliovirus using computer-generated design. *Proc Natl Acad Sci USA* 109(36):14301–14307.
- Brockmann H, Wimmer E (1965) Die konstitution des  $\epsilon$ -,  $\eta$ -,  $\xi$ -, und  $\zeta$ -rhodomycinons. *Chem Ber* 98:2797.
- Wöhler F (1828) Ueber künstliche bildung des harnstoffs. *Annalen der Physik* 88(issue 2):253–256.
- Stanley WM, Loring HS (1936) The isolation of crystalline tobacco mosaic virus protein from diseased tomato plants. *Science* 83(2143):85.
- Wimmer E, Reichmann ME (1968) Pyrophosphate in the 5'-terminal position of a viral ribonucleic acid. *Science* 160(3835):1452–1454.
- Wimmer E, Chang AY, Clark JM, Jr., Reichmann ME (1968) Sequence studies of satellite tobacco necrosis virus RNA. Isolation and characterization of a 5'-terminal trinucleotide. *J Mol Biol* 38(1):59–73.
- Wimmer E, Reichmann ME (1969) Two 3'-terminal sequences in satellite tobacco necrosis virus RNA. *Nature* 221(5186):1122–1126.
- Yogo Y, Wimmer E (1972) Sequence studies of poliovirus RNA. II. Polyadenylic acid at the 3'-terminus of poliovirus RNA. *Proc Natl Acad Sci USA* 69(7):1877–1882.
- Lee YF, Nomoto A, Detjen BM, Wimmer E (1977) A protein covalently linked to poliovirus genome RNA. *Proc Natl Acad Sci USA* 74(1):59–63.
- Kitamura N, et al. (1981) Primary structure, gene organization and polypeptide expression of poliovirus RNA. *Nature* 291(5816):547–553.
- Rothberg PG, Adler CJ, Kitamura N, Wimmer E (1980) VPg: The genome-linked protein of picornaviruses. *Bio-synthesis, Modification, and Processing of Cellular and Viral Polyproteins*, ed Koch G (Academic, New York), pp 309–319.
- Wimmer E, Paul AV (2010) The making of a picornavirus genome. *The Picornaviruses*, eds Ehrenfeld E, Domingo E, Roos RP (ASM Press, Washington, DC), pp 33–55.
- Kräusslich HG, Wimmer E (1988) Viral proteinases. *Annu Rev Biochem* 57:701–754.
- Jang SK, et al. (1988) Evidence in vitro for internal entry by the translational machinery in the 5' non-translated region of encephalomyocarditis virus RNA. *J Virol* 62:2636–2643.
- Jang SK, Davies MV, Kaufman RJ, Wimmer E (1989) Initiation of protein synthesis by internal entry of ribosomes into the 5' nontranslated region of encephalomyocarditis virus RNA in vivo. *J Virol* 63(4):1651–1660.
- Pelletier J, Sonenberg N (1988) Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature* 334(6180):320–325.
- Gromeier M, Lachmann S, Rosenfeld MR, Gutin PH, Wimmer E (2000) Intergeneric poliovirus recombinants for the treatment of malignant glioma. *Proc Natl Acad Sci USA* 97(12):6803–6808.
- Goetz C, Gromeier M (2010) Preparing an oncolytic poliovirus recombinant for clinical application against glioblastoma multiforme. *Cytokine Growth Factor Rev* 21(2-3):197–203.
- Mendelsohn CL, Wimmer E, Racaniello VR (1989) Cellular receptor for poliovirus: Molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. *Cell* 56(5):855–865.
- Gromeier M, Wimmer E (2003) US Patent 6,518,033.
- Molla A, Paul AV, Wimmer E (1991) Cell-free, de novo synthesis of poliovirus. *Science* 254(5038):1647–1651.
- Toyoda H, Yin J, Mueller S, Wimmer E, Cello J (2007) Oncolytic treatment and cure of neuroblastoma by a novel attenuated poliovirus in a novel poliovirus-susceptible animal model. *Cancer Res* 67(6):2857–2864.
- Wimmer E (2006) The test-tube synthesis of a chemical called poliovirus. The simple synthesis of a virus has far-reaching societal implications. *EMBO Rep* 7(spec no):S3–S9.
- Chumakov K, Ehrenfeld E, Wimmer E, Agol VI (2007) Vaccination against polio should not be stopped. *Nat Rev Microbiol* 5(12):952–958.
- National Research Council (2004) *Biotechnology Research in the Age of Terrorism* (National Academy Press, Washington, DC).
- Tumpey TM, et al. (2005) Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 310(5745):77–80.
- Coleman JR, et al. (2008) Virus attenuation by genome-scale changes in codon pair bias. *Science* 320(5884):1784–1787.
- Mueller S, et al. (2010) Live attenuated influenza virus vaccines by computer-aided rational design. *Nat Biotechnol* 28(7):723–726.