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The test-tube synthesis of a chemical called poliovirus

The simple synthesis of a virus has far-reaching societal implications

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In July 2002, newspapers across the globe reported that scientists had created a virus in a test tube. This unexpected news struck a raw nerve among lay people and scientists alike. The work was condemned as dangerous and irresponsible, scorned as a stunt and perceived as a challenge to divine power. It was also hailed as a milestone in biology. What really happened?

Guided by the nucleotide sequence, which my colleagues and I determined in 1981 (Kitamura *et al*, 1981), Jeronimo Cello, Aniko Paul and I described the chemical synthesis of a DNA molecule equivalent to the poliovirus genome (Cello *et al*, 2002). Using methods that we developed in the 1980s and 1990s (van der Werf *et al*, 1986; Molla *et al*, 1991), we then converted the virus-specific DNA by simple *in vitro* biochemical manipulations into authentic poliovirus particles (Cello *et al*, 2002). On paper, the synthesis looked simple and, not surprisingly, it was immediately predicted—correctly—that a similar method could be used to synthesize any virus, including smallpox.

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Poliovirus, which was discovered nearly a century ago by Karl Landsteiner and Erwin Popper (1909), is a human virus that replicates in the gastrointestinal tract after ingestion. In rare instances, the virus invades the central nervous system where it destroys motor neurons that control muscle movement. This results in a terrifying disease, called poliomyelitis, which leads to irreversible paralysis and even death (Mueller *et al*, 2005a). Although the virus is relatively benign—the ratio of infection to neurological complication ranges from 1:100 to 1:1,000—the speed with which it spreads caused frightening epidemics among unprotected populations during the first half of the twentieth century.

A

$C_{332,652}H_{492,388}N_{98,245}O_{131,196}P_{7,501}S_{2,340}$

B

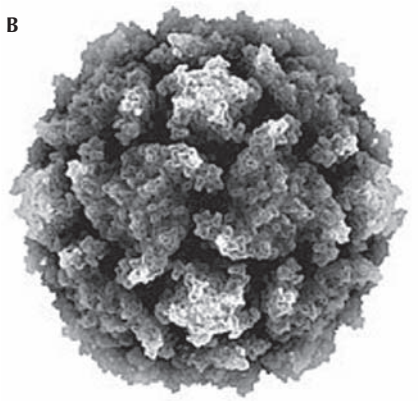

Fig 1 | Poliovirus and its empirical formula. (**A**) Empirical formula of the organic matter of poliovirus (Molla *et al*, 1991). (**B**) Depiction of a poliovirus particle generated from X-ray crystallographic data (Hogle *et al*, 1985).

The development of two vaccines, the inactivated poliovirus vaccine (IPV) of Jonas Salk in 1954 and the attenuated oral vaccine (OPV) of Albert Sabin in 1957, broke the terrifying grip of poliomyelitis in the mid-1950s. The distinct mode of oral administration and the mechanism of eliciting a protective immune response—both mucosal and humoral—made the OPV the favourite for mass vaccination. Encouraged by the reduction of poliomyelitis in the Americas, the World Health Organization (WHO; Geneva, Switzerland) started a global vaccination campaign in 1988 with the aim of eradicating poliovirus. So far, it has been a success: the global incidence of poliomyelitis decreased from more than 350,000 cases in 150 countries in 1988 to 1,255 cases in 18 countries in 2004 (Kew *et al*, 2005). In spite of the unexpected resilience of the virus (Heymann *et al*, 2005), there is great optimism among the officials conducting the global eradication campaign that all polioviruses will eventually be eliminated. Not everybody, however, shares this optimism. Moreover, the question arises as to whether a virus whose 'formula'—genome sequence—is known can ever be eradicated.

The empirical formula of poliovirus (Fig 1A; Molla *et al*, 1991) is $C_{332,652}H_{492,388}N_{98,245}O_{131,196}P_{7,501}S_{2,340}$. Because poliovirus is a quasi-species (Wimmer *et al*, 1993), the number of atoms in viral particles represents an average from a large population of different viruses. There might be little practical use in describing poliovirus by its empirical formula, but it persuasively portrays the virus as a chemical. Placing the atoms in order, a particle of high symmetry emerges (Fig 1B;

TTAAAACAGCTCTGGGGTTGTACCCACCCCAGAGGCCCACGTGGCGGCTAGTACTCCGGTATTGCGGTACCCTTGTACGCCTGTTTTATACTCCCTTCCCGTAACTTAGACGCACAAAACCAAGTTCAATAGAAGGGGG
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Fig 2 | Genome sequence of poliovirus, an RNA virus (Kitamura *et al*, 1981; Racaniello & Baltimore, 1981a), shown here in the corresponding DNA sequence.

Hogle *et al*, 1985), with all the properties required for its proliferation and survival in nature. These properties are encoded in the viral genome, a single-stranded RNA molecule of about 7,500 nucleotides (Fig 2; Kitamura *et al*, 1981).

At present, it is impossible to synthesize RNA of such length chemically. We therefore rerouted the synthesis through double-stranded DNA, using mail-ordered complementary oligonucleotides, which can be assembled in a linear fashion. After many steps of elongating the DNA strand by adding new oligonucleotides, we obtained a genome-length double-stranded complementary DNA (cDNA) of about 7,500 base pairs that contained all the genetic information of the viral RNA genome (Fig 3A). This synthetic cDNA was transcribed into viral RNA using a specific RNA transcriptase (van der Werf *et al*, 1986), thereby yielding infectious viral RNA (Fig 3A). We could have simply transfected this RNA into human cells to obtain authentic poliovirus; instead, we opted to violate the normal replicative cycle of the virus by mixing the RNA with a cell-free extract of uninfected human cells that was devoid of nuclei, mitochondria and other cellular organelles. As we showed a decade earlier (Molla *et al*, 1991), the RNA was both translated and replicated to form viral proteins and new genomes until a critical mass of viral products was reached; at this stage, poliovirus particles spontaneously assembled (Fig 3A).

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Our experiment has thus overthrown one axiom in biology—namely, that the proliferation of cells or, for that matter, viruses depends on the physical presence of a functional genome to instruct the replication process. It was believed that without parental genomes, no daughter cells or progeny viruses would arise. We have broken this fundamental law of biology by reducing poliovirus to a chemical entity, which can be synthesized on the basis of information stored in the public domain—an experimental proof of principle that is applicable to the synthesis of all viruses (Kitamura *et al*, 1981; van der Werf *et al*, 1986; Molla *et al*, 1991; Cello *et al*, 2002).

Indeed, any DNA molecule of any length could be assembled from oligonucleotides; thus, a virus might be resurrected from pure genomic information. We were probably among the first to realize the

potential dangers associated with this technology—the possible misuse of viral synthesis in bioterrorism. The US Defense Advanced Research Project Agency (Arlington, VA, USA) took the same stand and provided funding for our project, an endeavour we considered as a wake-up call. Indeed, the widespread attention generated by our publication raised the overall awareness of the new reality of synthetic viruses and its possible consequences.

Chemists have historically considered *de novo* synthesis to be the ultimate proof for any deciphered chemical structure. If the synthetic product, such as an antibiotic, had the same properties as the natural isolate *in vitro* and *in vivo*, the structure was considered proven. For the chemist, the synthesis of authentic poliovirus provides proof that the sequence originally deciphered from genomic RNA is correct (Cello *et al*, 2002; Kitamura *et al*, 1981; Racaniello & Baltimore, 1981a). Although nobody really doubted the accuracy of the poliovirus genome sequence because it had been determined multiple times, there are cases in which DNA synthesis might be the only way to ascertain that a genome sequence is correct, as in the resurrection of the Spanish influenza virus from archaeological samples (Tumpey *et al*, 2005; see below).

The synthesis of a replicating 'organism' in the absence of a natural template was without precedent at the time of its publication in 2002, and provoked unusual and widespread responses. Leaving the scientific aspects aside, there were two factors that we believe contributed to the emotional, and sometimes contradictory, reactions. The first was the format in which the manuscript was presented to the public. During the editing process, our paper was stripped bare of our original discussion of the ethical and societal implications. In fact, we lost a battle with the editors of *Science* and agreed to a final text that resembled a laboratory report. The importance of how scientific data reach the public should not be underestimated; in our case, the brevity led commentators to twist the story in any desired direction—sometimes inflammatory and of little substance—and left us, the authors, with little defence. The second reason was the timing of the publication. The manuscript appeared less than a year after the terrorist attacks on 11 September 2001 and the anthrax bioterrorist attacks in 2001. Consequently, news

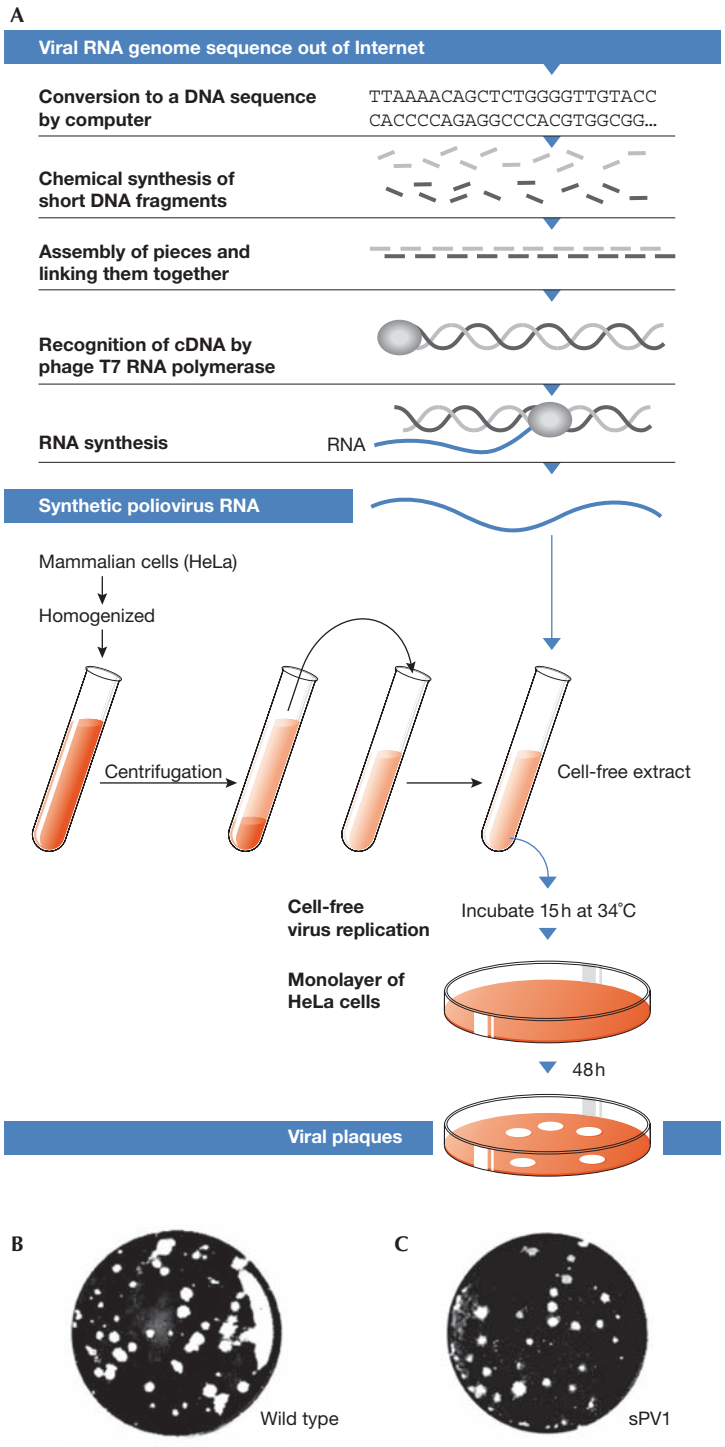

Fig 3 | Synthesis of poliovirus in the absence of natural template. (**A**) Short complementary segments of synthetic DNA (oligonucleotides) are annealed, and enzymatically extended and ligated (connected). A full-length complementary DNA (cDNA) is assembled stepwise to represent the entire genetic information of the poliovirus RNA genome in the form of DNA. The cDNA is then transcribed into infectious viral RNA by a T7 RNA transcriptase. This RNA is used to seed a HeLa cell-free extract that will replicate, just like in intact cells, to form progeny virions (Cello *et al*, 2002; Molla *et al*, 1991). (**B**,**C**) Evidence for *de novo* synthesized virus is provided by plaque assays. Poliovirus plaques derived from synthetic virus (sPV1) and wild-type virus, respectively, are formed on monolayers of HeLa cells (Cello *et al*, 2002). Reproduced from Mueller *et al* (2005b), with permission.

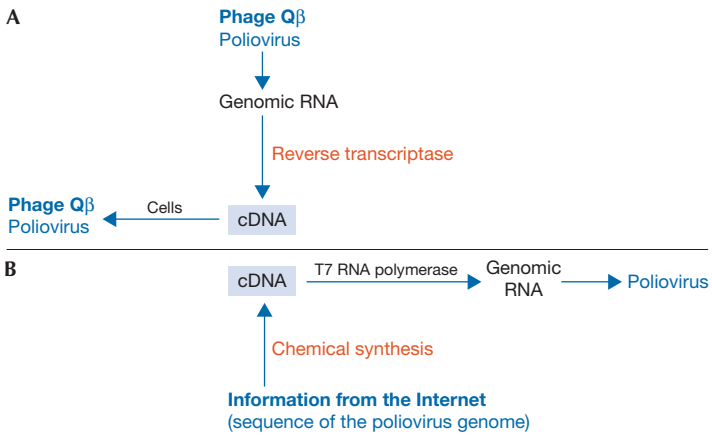

Fig 4 | Two different strategies for generating RNA viruses via complementary DNA (cDNA) intermediates. (**A**) Synthesis of cDNA catalysed with reverse transcriptase followed by transfection of the cDNA into host cells (Taniguchi *et al*, 1978; Racaniello & Baltimore, 1981b). (**B**) Chemical synthesis of cDNA followed by transcription and incubation of the infectious viral RNA in a cell-free extract (Cello *et al*, 2002; Molla *et al*, 1991).

of the poliovirus synthesis reached a highly irritated public, particularly in the USA, which was ready to link it with bioterrorism. The US public worried that our synthesis provided a blueprint for generating dangerous biological weapons that might endanger national security.

The different reactions to the poliovirus synthesis were astounding and perplexing. In general, they fell into one of the following categories: positive reactions, ethical concerns, questions about the scientific value of the experiment, concerns about jeopardizing the global eradication of poliovirus, issues of national security, and issues of freedom and censorship of biological research. Thankfully, the majority of responses were positive.

Considering ethical concerns, our research has fuelled a long-standing debate on whether viruses are alive. So far, the relationship of viruses to life has been described in vague terms at best: "viruses are parasites that skirt the boundaries between life and inert matter," said Luis P. Villarreal; "viruses lead a kind of borrowed life," Marc H. V. van Regenmortel and Brian W. Mahy maintained; and "whether or not viruses should be regarded as organisms is a matter of taste," according to André Lwoff (Villarreal, 2004). However, these descriptions are inconclusive, as humans have not reached a consensus on how to define life, and will probably never agree on a single definition. Not surprisingly, there has been a deluge of different definitions of life formulated by individuals of every province in society, including scientists, philosophers and theologians. A quote in 1975 by John Maynard Smith, favoured by many scientists including myself, describes life "as any population of entities which has the properties of multiplication, heredity and variation" (Lahav, 1999). Viruses qualify for this classification. By contrast, viruses have been excluded from classification as living entities because they do not consume 'food' or produce 'waste', because they lack metabolism and, as intracellular parasites, because they depend upon a supply of energy. Robert Hazen, however, has argued that "such a taxonomic question [that is, life versus non-life] is fruitless given the likely sequence of steps of increasing complexity that must have characterized the transition from a geochemical to a biochemical world" (R. Hazen, personal communication; Hazen, 2005).

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When I am asked whether poliovirus is a non-living or a living entity, my answer is yes. I regard viruses as entities that alternate between non-living and living phases. Outside the host cell, poliovirus is as dead as a ping-pong ball. It is a chemical that has been purified to homogeneity and crystallized (Schaffer & Schwerdt, 1955), with its physical and chemical properties largely determined (Wimmer *et al*, 1993), and its three-dimensional structure solved. Just like a common chemical, poliovirus has been synthesized in the test tube.

Once poliovirus, the chemical, has entered the cell, however, it has a plan for survival. Its proliferation is then subject to evolutionary laws: heredity, genetic variation, selection towards fitness, evolution into different species and so forth—that is, poliovirus obeys the same rules that apply to living entities. One could even argue that poliovirus undergoes sexual reproduction in the infected cell, as it readily recombines with sibling progeny or with other related viruses (P. Jiang, J.A.J. Faase, H. Toyoda, A.E. Gorbalenya and E. Wimmer, unpublished data) to exchange genetic information (Wimmer *et al*, 1993).

Barring religious beliefs, scientific wisdom holds that living entities will irreversibly die. I suggest that viruses do not follow this destiny, but rather switch between non-living and living phases. These seemingly incompatible qualities of viruses might be difficult to comprehend, but think of the electron: it took physicists decades to accept that it is both a wave and a particle.

In 1828, Friedrich Wöhler, a German chemist, shattered the doctrine of vitalism by synthesizing an organic compound (urea) from an inorganic compound (ammonium cyanate; Wöhler, 1828). Vitalism states that organic compounds possess properties that cannot be explained in physical or chemical terms. Similarly to the synthesis of urea, no 'vital force' was necessary to 'instruct' the poliovirus genome during the chemical synthesis and transcription of the cDNA *en route* to infectious viral RNA. There is nothing transcendental about the sequence of the poliovirus genome shown in Fig 2. We have concluded that vitalism is not necessary to explain the properties of poliovirus or any virus (Cello *et al*, 2002).

It must be emphasized, however, that we have merely reproduced poliovirus by following the blueprint of the viral genome (Kitamura *et al*, 1981). We did not 'create' this virus. The complexity of virus structure and replication currently makes it impossible to design an entirely new virus, regardless of whether the hosts are bacteria, plants or mammals.

I understand that my view of poliovirus as an inanimate chemical with a life cycle is

not uniformly shared. It might be criticized for adopting a "reductionist approach limiting our scientific understanding of living organisms" (Cho *et al*, 1999). Religious considerations might also override my position on viruses and life. I do not contest the divergent views of others, and I expect that my views—and our scientific data—will be equally respected. Avoiding dialogues or even ignoring ethical issues can lead not only to misunderstandings but also to communal upheaval and violence.

In 1978, Charles Weissmann and colleagues revolutionized RNA virology by inventing reverse genetics (Taniguchi *et al*, 1978). To use the methods developed for DNA molecular biology, the researchers converted the purified genomic RNA of phage Qβ, a virus of bacteria, into full-length cDNA with the enzyme reverse transcriptase. This virus-specific cDNA yielded authentic RNA Qβ phages after transfection into bacteria (Fig 4A). After 3 years, Racaniello & Baltimore (1981b) repeated this experiment using purified poliovirus RNA and human cancer cells as hosts (Fig 4A). They too obtained authentic virus. After our publication of synthetic poliovirus in 2002, the question was asked: why bother to chemically synthesize cDNA (Fig 4B) when this can be done faster and much more cheaply with the help of enzymes? In relation to this, a perplexing view was aired in the journal *Science*, claiming that the chemical synthesis of poliovirus cDNA was just a publicity stunt (Block, 2002).

> The emerging scientific and logistic difficulties of poliovirus eradication, combined with the new reality of rapid *de novo* synthesis of viruses, force us to ask whether the polio campaign has been rendered a dream

Critics in this frame of mind ignore the fact that generating poliovirus cDNA *per se* was not the message of our 2002 paper. A major point was that viruses can be looked at as chemicals and, accordingly, can be synthesized from publicly available information with off-the-shelf chemicals. Notably, the entire process of recreating the virus can happen outside living cells. It should also be mentioned that we predicted in 2002 that, given the rapid progress in biotechnology, it would soon be possible to synthesize poliovirus in a few days. As I will discuss later, the future has already begun.

Finally, Block also argued that the poliovirus synthesis was scientifically worthless (Block, 2002). Compared with the sequence of the wild-type genome, the synthetic cDNA had 27 base changes that were intentionally placed into the nucleotide chain as genetic markers. These base changes had no influence on the replication of the virus in HeLa cells (Fig 3B,C). However, one of the mutations attenuated the neurovirulence of the synthetic virus 10,000-fold in transgenic mice susceptible to poliovirus infection (de Jesus *et al*, 2005). This surprising result has opened a new avenue of studies in our laboratory. Although some critics claimed that we did not learn anything, the poliovirus synthesis in fact yielded valuable scientific information about the genetics of viral pathogenesis. Indeed, our experience of synthesizing poliovirus has inspired us to pursue viral genome synthesis for medical applications, for example, to explore novel strategies in vaccine development (S. Mueller, D. Papamichail, J.R. Coleman, S. Skiena, E. Wimmer, unpublished data).

Global eradication of an infectious agent is the best way to control the disease it causes. The unprecedented success of the smallpox eradication campaign encouraged the WHO to eliminate poliomyelitis by eradicating poliovirus. As mentioned above, the world population is now better protected against poliomyelitis than ever before. Therefore, the poliovirus synthesis published in 2002 presents no health threat whatsoever.

However, a different question arises: does the test-tube synthesis negate efforts to eradicate poliovirus? The conceptual answer to this is yes. Poliovirus cannot be declared extinct because the sequence of its genome is known and modern biotechnology allows it to be resurrected at any time *in vitro*. This is true for all viruses, including smallpox. Indeed, the global eradication campaign for polioviruses, now in its eighteenth year, has proven much more difficult than anticipated. Apart from the resilience of circulating wild-type viruses, major problems have emerged as a result of intrinsic properties of the OPV. It has the propensity to escape its designated role as a protecting immunogen by circulating in poorly immunized populations, thereby evolving into highly neurovirulent poliovirus strains after recombination with other enteroviruses (Kew *et al*, 2005; P. Jiang, J.A.J. Faase, A.E. Gorbalenya and E. Wimmer, unpublished data). This independent occurrence in different parts of the world causes yearly outbreaks of poliomyelitis. In addition, immune-deficient persons receiving the OPV can develop persistent infections, shedding highly neurovirulent poliovirus for years (MacLennan *et al*, 2004). The known number of persistently infected persons is small and the actual number of poliovirus shedders cannot be determined at the present time. But persistently infected individuals pose a serious health threat once vaccination has been terminated. These complications have led a panel of experts to recommend the development of novel anti-polio drugs for the control of poliomyelitis (National Research Council, 2006).

> Evidently, in late 2005, the general public was much better prepared, and perhaps better educated, to accept the rewards of rapidly advancing medical technologies without emotional outbursts

The WHO's current strategy calls for cessation of OPV vaccination 3 years after the last incidence of poliovirus-caused poliomyelitis. The risks inherent in this strategy are immense. Herd immunity against poliomyelitis will rapidly decline as new children are born who have not been infected with wild-type viruses or were not vaccinated, a condition that has never existed in human history. Thus, any outbreak of poliomyelitis will be disastrous, whether it is caused by residual samples of virus stored in laboratories, by vaccine-derived polioviruses or by poliovirus that is chemically synthesized with malignant intent. The emerging scientific and logistic difficulties of poliovirus eradication, combined with the new reality of rapid *de novo* synthesis of viruses, force us to ask whether the polio campaign has been rendered a dream. Our resources are perhaps better spent on controlling poliomyelitis rather than eliminating its cause. It has been suggested that vaccination against poliomyelitis, based on newly developed vaccines, might have to continue indefinitely (Agol *et al*, 2005).

Within days of the online publication of the synthesis of poliovirus in *Science* in July 2002, harsh comments from some scientists and politicians suggested that, for reasons of national security, the work was irresponsible and the manuscript should never have been published. Of course, it was certain then, and still is today, that the chemical synthesis of poliovirus does not pose a threat to the general population. Yet the question persists as to whether the poliovirus synthesis was a blueprint for bioterrorists.

> …it is even more urgent to develop new strategies in order to protect us from misuse by fostering open research in the broadest sense, not by restricting it

Bioterrorism relies mostly on infectious agents. Defence against these agents rests principally on research aiming to limit the impact of a harmful agent through either novel drugs or new vaccines. The free flow of information is considered essential to nurture scientific research. Therefore, it might be concluded that free dissemination of biological information fosters such progress and, thus, is the best strategy to guard against bioterrorism. Some critics consider this view as naive. It was the purpose of a workshop on Scientific Openness and National Security, organized by the US National Academy of Sciences and the US-based Center for Strategic and International Studies in Washington, DC, in January 2003, to initiate a broad discussion of these pressing issues. Delegates included editors of scientific journals, authors of papers in the biological sciences, members of the US government, secret service personnel and journalists. Their deliberations led to guidelines for journal editors and authors that assured publication of scientific information, but also proposed measures guarding against dissemination of highly sensitive, perhaps dangerous, information (Atlas *et al*, 2003; Kennedy, 2003).

However, all methods used for the synthesis of poliovirus were published long before the experiment was conceived. Thus, we neither described new technologies to synthesize DNA nor invented novel methods to convert cDNA into infectious viral RNA. The purposes of the poliovirus synthesis—namely, to establish a proof of principle and to sound a wake-up call—were accepted as reasonable at the 2003 workshop. Yet, should biological research in general be monitored to safeguard against misuse?

In October 2003, a Committee on Research Standards and Practices to Prevent the Destructive Application of Biotechnology, assembled by the US National Research Council, proposed a self-policing system for scientists to screen research that could potentially harm national security (National Research Council, 2004). The committee concluded that "the challenge is for the scientific community to develop a system that permits fundamental research to proceed unimpeded, while identifying research with great potential for misuse." While backing the freedom of journal editors to publish federally funded fundamental research within the guidelines developed in January 2003, the report suggests an expansion of the responsibility of Institutional Biosafety Committees at universities and research institutions. These committees will review research proposals and, if necessary, refer questionable projects to the Recombinant DNA Advisory Committee of the National Institutes of Health (Bethesda, MD, USA).

> …it was certain then, and still is today, that the chemical synthesis of poliovirus does not pose a threat to the general population

Ultimately, the outcome of an experiment in fundamental research in biology, perhaps in all sciences, cannot be predicted. Biological systems are too complex to be able to account for all variations, no matter how carefully the experiment is designed. Experiments with viruses are no exception. Intuition and creativity play as great a role in science as they do in the arts, but are no guarantee of success. Thus, even the best scientists suffer frequent humiliation when their experiments do not yield the expected results. However, unanticipated results are not solely disappointments but have also led to landmark discoveries that have transformed an entire discipline or spawned new technologies. Indeed, unanticipated results might have revolutionized the practice of medicine—consider the serendipitous discovery of penicillin, for example.

These considerations explain why it is difficult to separate research projects that potentially pose a danger to society from the overwhelming majority of studies from which we draw benefits, including added security. Ultimately, scientists themselves must take the responsibility for evaluating the risks inherent in their research. However, standards of biological research must be accepted globally. The benefits are few if such recommendations, as outlined above, are adhered to only in the USA.

Eighteen months after the poliovirus synthesis was published, a paper appeared describing the *de novo* synthesis, in just 2 weeks, of the 5,386 base-pair DNA genome of bacteriophage ϕX174 (Smith *et al*, 2003). The unedited DNA was transfected into bacteria, which sorted the good from the bad and produced viable phages. This rapid assembly of DNA was a technical feat that could be applied without modification to any virus, including those on the select bioterrorist agents list from the US Centers of Disease Control and Prevention (Atlanta, GA, USA). Thus, the strategy could be used to synthesize poliovirus or Ebola virus within weeks. Surprisingly, this fact was lost on the American public in December 2003; the great uproar after the poliovirus synthesis in 2002, which had been largely fuelled by Craig Venter, senior author of the 2003 ϕX174 paper, had been mostly forgotten.

Another landmark publication in virology was the resurrection of the Spanish influenza virus by chemical synthesis (Tumpey *et al*, 2005). This virus, with the genetic signature H1N1, caused the horrific influenza pandemic of 1918/1919, which killed an estimated 20–50 million people worldwide. Given the constant danger of new influenza pandemics, including the uncertain threat of the highly pathogenic avian influenza H5N1 strain, it was deemed important to resurrect the Spanish influenza virus and to decipher the molecular mechanisms by which it expressed its deadly instincts.

A tentative genome sequence of the Spanish influenza virus was deciphered from specimens uncovered eight decades after the pandemic. This sequence guided the *de novo* synthesis of the deadly virus, which, in turn, permitted the study of its pathogenesis (Tumpey *et al*, 2005). The publication of the resurrected killer influenza, which caused

much public concern, was carefully embedded in multiple layers of commentaries, all eventually supporting its synthesis and publication. It was argued that the benefit of the scientific endeavour, which yielded numerous important new insights into influenza pathogenesis, outweighed the risks of misuse. I share this view. Evidently, in late 2005, the general public was much better prepared, and perhaps better educated, to accept the rewards of rapidly advancing medical technologies without emotional outbursts.

Just over a year earlier, another paper exemplified how fast the technology in DNA synthesis was progressing (Tian *et al*, 2004). The authors described a new method by which they claimed that any DNA molecule of 20,000 base pairs could be synthesized at a price of US$1. If so, the dreaded hepatitis B virus or poliovirus could be synthesized for a few cents and Ebola virus for a few dollars. This new reality in synthetic biology was not unexpected, but the speed with which it arrived was astounding. Thus, it is even more urgent to develop new strategies in order to protect us from misuse by fostering open research in the broadest sense, not by restricting it.

Viruses have been defined as obligatory intracellular parasites in need of a living cell for replication. Our work challenges this definition. After the sequence of the poliovirus genome was determined, and a protocol for the *de novo* test-tube replication of the virus in a cellular extract was developed, the chemical synthesis of the genome was a logical step to assert its character as a chemical. For many virologists, the dual nature of viruses as chemicals with formulae stored in data banks and as organisms circulating in nature was not news. To most scientists and lay people, however, the reality that viruses can be synthesized was surprising, if not shocking. Then and now, we consider it imperative to inform society of this new reality, which bears far-reaching consequences.

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