R E S E A R C H

Chemical synthesis of a virus: the science and its implications

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Modern biology, also called molecular biology, biotechnology or genetic engineering, has witnessed many technological and conceptual advances over the past two decades. The foremost among these have been the Human Genome Project that enabled sequencing of not just our own genetic material, but also the genomes of many micro-organisms. However, as with any language, knowing the alphabet and being able to read it does not necessarily mean that we understand this genetic language. The next big step is to understand what our genes actually do.

The sequencing of genomes has thrown up many ethical, social and legal issues relating to patenting (ownership) of genetic material and information, and accessibility of this information.

Science in its purest form is value-neutral, but technologies that develop out of this knowledge depend upon the intent of the user. It is the society, the scientific establishment and the commercial interests that decide how to use that technology. Nuclear technology can be used either for public good by providing an efficient source of energy, or for large scale destruction in the form of nuclear bombs. Biotechnology will be no different. While it is being used to find novel cures and vaccines for many diseases, it also has the potential to create novel (and dangerous) pathogens with devastating consequences.

This point was driven home recently by the first chemical synthesis of a human pathogen, in this case poliovirus (1).

Chemical synthesis of poliovirus

Many viruses, including poliovirus, are simple consisting of some genetic material (RNA or DNA) surrounded by a protein coat. The genetic material instructs the virus-infected cells to produce more viruses. So, if one were to produce the viral RNA or DNA and put it in a proper cell, new viruses would be produced. This has been accomplished for many viruses, using genetic material derived from a natural virus. The recent work has gone a step further by chemically synthesising the viral genome, thus obviating the need for a template derived from a natural virus.

The poliovirus genome consists of 7500 chemical units (bases or nucleotides) of RNA. Since chemical synthesis of RNA is far more difficult than DNA, the complete poliovirus genome was first assembled as a DNA copy. This was accomplished by stitching together chemically synthesised pieces of DNA (called oligonucleotides), using a routine enzyme-driven in vitro process. The chemical synthesis of oligonucleotides is also a standard and automated process that is used by thousands of laboratories the world over. Further, these short pieces of DNA can be ordered from any of the numerous mail-order companies for as little as 40-50 cents (about Rs. 20-25) per base.

The DNA was then converted into RNA in a test tube using a commercially available enzyme. This RNA, which would be identical to poliovirus genomic RNA, was then put into a mixture of proteins derived from cells. Such a mixture of proteins capable of supporting the replication of poliovirus RNA and generation of new virus particles has been known since the early 1990s. In this cell-free protein system, the synthetic poliovirus RNA produced viral proteins that were identical to those produced by poliovirus-infected cells. Further, when incubated with intact cells, the mixture was able to form plaques indicative of viral infection that were identical to those produced by natural poliovirus. In further tests to determine specificity and pathogenic nature of the synthesised virus, it was neutralised by poliovirus-specific immune sera and produced neurovirulence in a transgenic mouse model of poliovirus infection.

Thus, synthetic poliovirus genetic material was able to produce viable virus that was identical to natural poliovirus. This was accomplished without using either a template or an intact living cell. This is the first example of chemical synthesis of a virus from scratch.

Is such work necessary?

This is a question society will have to consider, just as it did in the '70s when recombinant DNA technology was discovered and applied, or very recently when Dolly was cloned making way for possible cloning of human beings. Craig Venter, who led the private effort to sequence the human genome, says "I think it's inflammatory, without scientific justification. To purposely make a synthetic human pathogen is irresponsible" (2). He adds that "this work should never have been done, funded, or published; somehow the whole system broke down here." (3)

There are more immediate questions that we should address. Did this work teach us something we did not already know? Does it extend the frontiers of science? Some would say no because we already knew that oligonucleotides can be assembled into genes, DNA can be transcribed in vitro into RNA and that poliovirus RNA can produce virus particles when incubated with an appropriate cell extract. The present work has simply put the entire recipe together. However, others would argue that this is conceivably the first chemical

synthesis (creation) of life in a test tube. Given the current state of knowledge, this work is not insightful enough to be published in a major scientific journal which claims to publish only paradigm-shifting findings.

Implications for public health

The 1988 World Health Assembly resolved to eradicate poliovirus from the globe by the year 2000, making this the second human pathogen after smallpox targeted for eradication. Due to sustained global immunisation programmes, this goal has largely been achieved with 99% of the world being declared polio-free in 2000 (4).

Can polio immunisation ever be discontinued? Analogous to the strategy used after smallpox eradication, it is planned to stop polio immunisations a few years after its eradication. However, in view of this new research, can we ever afford to do that? Vaccinations might still be needed to protect against use of synthetic poliovirus as a bioweapon. "This just says we're going to have to sustain some immunisation for the indefinite future", says D A Henderson, principal adviser on public health preparedness to the U S government (2).

Would poliovirus make a good bioweapon? Probably not. A good bioweapon would be distributed through aerosols, have high rates of infectivity and mortality, and the target population should be immunologically naïve to it. Poliovirus does not satisfy these criteria. It is transmitted through food, only 0.1-0.5% of exposed people suffer paralysis and most of the world's population has been vaccinated against it. However, viruses such as Ebola and smallpox fit this bill perfectly. While technology does not exist today to chemically synthesise these more complicated viruses, no one anticipated ten years ago that poliovirus could be synthesised from scratch. Barry Bloom, Dean, Harvard School of Public Health comments: "This should really raise some red flags. It means that more complicated viruses can be created – and that it is also possible to create viruses that do not exist in the wild" (3). The real value of this work would be in helping public health systems to be realistic about assessing future threats.

The practice and reporting of biomedical research

The ability to freely report and exchange information has been a crucial part of public health. Shall we still enjoy the freedom to do so?

"Can scientific journals become cookbooks for terrorists by providing easy recipes for assembling germs that could be unleashed easily on people?" asks an article in a national daily (5). In the post-September 11 world such questions will be asked. The same article quotes the respected British medical journal *Lancet*, "Advances in microbiology can eventually facilitate cures for diseases, but a dedicated terrorist can figure out how to use that same information for harm" (5). Consequently, reviewers have been asked to alert editors should they find potentially problematic material in the papers they are reviewing. The U S House of Representatives is looking at a resolution that asks editors of scientific journals to 'exercise restraint' in publishing such material (5). A more appropriate request should have been made to the United States Defense Department, which funded this research, to exercise caution in funding such research.

Dr Wimmer is quoted as saying "If it is not done by sensible scientists, then it will be done by terrorists." (3) What arrogance! Would this science still be 'sensible' if it was done outside of the United States, let's say in India or Brazil? However, biomedical scientists working in countries such as India may face problems in freely conducting their research. The CEO of Integrated DNA Technologies, the company that synthesised the oligonucleotides for the polio research, is quoted as saying that they are now considering checking sequences ordered by customers against a database of pathogens (2).

I am a virologist working on hepatitis viruses and HIV and I routinely mail-order oligonucleotides for my work. Will I now be considered a potential terrorist who may be synthesising these viruses in my backyard? The real danger is from the hype that such work is able to generate. This may then be used by vested interests to create national or regional barriers in the practice of science.

Conclusion

The reported chemical synthesis of a human pathogen has raised many questions that society as a whole and the biomedical community in particular, will have to address. Is this going to be the route to new kinds of terrific genetically engineered bioterror, or is this just a stunt? Will it help the world be realistic about assessing future threats, or is it going to push an already paranoid world into creating barriers for research and advocating censorship of publications? Many of these issues will have to be addressed sooner than later.

References

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