

# CURRENT SCIENCE

Volume 85 Number 11

10 December 2003

## EDITORIAL

### Synthesizing Life

*'I stated that by the action of cyanogen on liquid ammonia, besides several other products, there are formed oxalic acid and a crystallizable white substance, which is certainly not ammonium cyanate, but which one always obtains when one attempts to make ammonium cyanate by combining cyanic acid with ammonia e.g. by so-called double decomposition. The fact that in the union of these substances they appear to change their nature, and give rise to a new body, drew my attention anew to this subject, and research gave the unexpected result that by combination of cyanic acid with ammonia, urea is formed, a fact that is noteworthy since it furnishes an example of the artificial production of an organic, indeed a so-called animal substance, from inorganic materials.'*

Friedrich Wöhler  
*Ann. Phys. Chem.*, 1828, **88**, 253

*'The chemical synthesis of the viral genome combined with de novo cell-free synthesis has yielded a synthetic virus with biochemical and pathogenic characteristics of poliovirus. In 1828, when Wöhler synthesized urea, the theory of vitalism was shattered. If the ability to replicate is an attribute of life, then poliovirus is a chemical,  $C_{332,652}H_{492,388}N_{98,245}O_{131,196}P_{7501}S_{2340}$  . . . , with a life cycle.'*

J. Cello, A. V. Paul and E. Wimmer  
*Science*, 2002, **297**, 1016

*'The capabilities of DNA synthesis have lagged far behind our ability to determine sequences during the past 30 years. If this gap can be closed, then limitless possibilities for the application of synthetic methods to the study and practical application of genomics will emerge. . . . Synthetic genomics will become commonplace and provide the potential for a vast array of new and complex chemistries, altering our approaches to production of energy, pharmaceuticals and textiles.'*

H. O. Smith *et al.*  
*Proc. Natl. Acad. Sci. USA*, 2003, Dec. 2

Wöhler's urea synthesis marked a decisive turning point in the history of chemistry. The laboratory production of a substance that had thus far been associated exclusively with living organisms breached the barrier between

the animate and the inanimate. Wöhler and, a few decades later, Pasteur delivered the death blows to the theory of vitalism. No mysterious 'life force' appeared to be necessary for maintaining living cells; chemistry and biochemistry seemed sufficient. Wöhler was exuberant when he wrote of his experiments to Jöns Jacob Berzelius: 'I can no longer as it were, hold back my chemical urine; and I have to let out that I can make urea without needing a kidney, whether of man or dog; the ammonium salt of cyanic acid is urea'. Berzelius had, a year earlier, in 1827 noted in his *Textbook of Chemistry*: 'In living Nature the elements seem to obey entirely different laws than they do in the dead. . . . The essence of the living body consequently is not founded in its inorganic elements, but in some other thing, which disposes the inorganic elements. . . to produce a certain result, specific and characteristic of each species' (Hunter, G. K., *Vital Forces*, Academic Press, 2000, p. 56). We know today that urea is an exceedingly simple substance; its chemical formula  $CH_4N_2O$  would not appear intimidating even to a high school student. But even in Wöhler's time urea was not regarded by some as a 'real organic molecule', with Johannes Muller sounding magisterial in his dismissal: 'Urea, however, can scarcely be considered as organic matter, being rather an excrement than a component of the animal body'.

In the century after Wöhler, chemistry and life remained resolutely separate, with the rapid and divergent growth of the disciplines of organic chemistry and biochemistry. At times it almost seemed that any discussion of living organisms and life must have been banned in departments of organic chemistry, in response to edicts issued by the high priests of the discipline. The chemical synthesis of proteins, nucleic acids and oligosaccharides was not an activity looked upon with favour by traditional departments of organic chemistry; the monotonous chemistry of the biopolymers did not seem to afford a sufficient intellectual challenge for the synthetic chemist. The formidable technical obstacles to the synthesis of biopolymers were dramatically overcome in the early 1960s, with the introduction of solid phase synthesis, first applied to peptides and proteins by R. B. Merrifield. DNA synthesis was achieved in a monumental effort by Har Gobind Khorana, with the first gene synthesis reported in the late 1970s. The introduction of solid phase procedures and

automation brought the synthesis of oligonucleotides, small DNA fragments, to molecular biology laboratories by the 1980s. But the methods have been restricted to assembling individual genes, by enzymatic joining (ligation) of synthetic nucleotide fragments. Genomes, which contain several genes strung together in sequence provide a more formidable synthetic challenge. If 'life' must be synthesized, then genomes which provide the blueprint for an organism's chemistry are the target of choice.

One hundred and seventy four years after Wöhler, in a chemical *tour de force*, Eckard Wimmer and his colleagues at the State University of New York, Stony Brook reported the synthesis of poliovirus complementary DNA and the successful generation of an infectious viral particle in the 'absence of any natural template'. The genome length of ~ 7500 nucleotides may be compared with the first gene synthesized in the late 1970s, which had a length of 207 nucleotides. While the methodologies for gene assembly have been in place for some years, the synthesis of the poliovirus demonstrates that a virus capable of replication, and therefore a life form, can be put together by laboratory procedures without the intervention of any native viral DNA (Cello, J. *et al.*, *Science*, 2002, **297**, 1016). In a more recent report Craig Venter and his colleagues report the construction of a synthetic genome of the bacteriophage phiX174, which is a virus capable of infecting the common bacterium *E. coli*. The assembly of a stretch of 5386 nucleotides was achieved in a remarkable time of 14 days; improved methodologies enhancing the fidelity of synthesis. Once again infective viral particles could be generated. These two examples suggest that methodologies now exist for the assembly of larger genomes, permitting the laboratory creation of organisms with 'minimal genomes'. Chemistry and biochemistry have now seamlessly integrated to permit the synthesis of 'life'.

Viruses live in a shadowy no-man's land on the borders of chemistry and biology. The simpler viruses, phiX174 and poliovirus among them, consist of relatively simple protein coats packaging small genomes, which possess the instructional capability for recruiting host machinery for viral replication. Many viruses, including the poliovirus have been crystallized and their atomic arrangements laid bare by X-ray diffraction, a technique that had its first major success in the early years of the 20th century when the Braggs, father and son, described the structure of sodium chloride. Viruses are not free-living creatures but need the resources of a host organism to multiply; nevertheless they are undoubtedly the simplest collection of chemicals that possess some of the many attributes of life. The work of Wimmer, Venter and their colleagues is a tribute to the robustness of the methodologies of chemistry and biochemistry, which have now brought us to the threshold of the synthesis of small genomes. Chemical synthesis, with assistance from enzymatic ligation and the polymerase chain reaction appears to be a viable strategy for assembling genomes in the laboratory, raising possi-

bilities that have so far been confined to the realm of science fiction. Critics have been quick to attack the Wimmer paper on the polio virus; arguing that similar methods may be used to put together dangerous viruses as weapons in the area of biological warfare (bioterrorism, seems a more widely used word, nowadays). Some biologists have gone so far as to suggest restrictions on the availability of viral genome sequences; a knee-jerk reaction which may hamper research in virology rather than protect dangerous exploitation of sequence information. Interestingly, the poliovirus paper was a blandly written technical report that offered no real justification for the choice of a human pathogen and made no reference to any ethical questions that may accompany this undoubted advance of scientific methodology. In the phiX174 paper that followed, Venter and his colleagues note: 'Prior to attempting synthesis of a microbial chromosome we commissioned an independent bioethical review of our proposed scientific plan. After more than one year of deliberation the reviewers concluded that we were taking a reasonable scientific approach to an important biological question. The broader implications of the creation of life in the laboratory can now be considered as a realistic possibility' (Smith, H. O. *et al.*, *Proc. Natl. Acad. Sci. USA*, 2003, Dec. 2). In the ethical review report entitled 'Ethical considerations in synthesizing a minimal genome', the authors note that 'the attempt to model and create a minimal genome represents the culmination of a reductionist research agenda about the meaning and origin of life that has spanned the 20th century'. They conclude decisively: 'The prospect of constructing minimal and new genomes does not violate any fundamental moral precepts or boundaries, but does raise questions that are essential to consider before the technology advances further' (Cho, M. K. *et al.*, *Science*, 1999, **286**, 2087).

Two complementary approaches to creating minimal life forms in the laboratory have emerged over the last few years. Downsizing an existing genome, a 'top-down' approach by large scale gene knock outs is already on the cards. The 'bottom-up' strategy with genome synthesis as the starting point is illustrated by the work on poliovirus and phiX174. But there is a long way to go before 'life' in its fullest form can be created artificially. The backdrop of evolution and selection makes living organisms an incredibly formidable target for chemical assembly. When asked to comment on origins of life research, Lynn Margulis was forthright: 'Biochemical systems are effectively historical accumulations. So I don't think there is ever going to be a packaged recipe for life; add water and mix and get life' (Horgan, J., *The End of Science*, Broadway Books, New York, 1996, p. 140). Whole genome synthesis promises a new approach. The 21st century may witness tumultuous advances on this front, paralleling the changes that began in the 19th century with Wöhler's serendipitous synthesis of urea.

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