A look back: Changes for the better

"What sort of molecule could function as a specific mutagen?" pondered Joshua Lederberg in his 1958 Nobel lecture. "How could this be generally accomplished except by another molecule of conforming length and periodicity, that is an analogous polynucleotide?" This genetics luminary recognized the nature of the problem but the absence of techniques for gene sequencing and the generation of complimentary oligonucleotides remained a serious obstacle, and Lederberg concluded that "pending such advances, *specific* mutagenesis is an implausible expectation." Fortunately, this would not remain the case for long.

In 1971, Clyde A. Hutchison III was a newly appointed faculty member at the University of North Carolina, studying the single-stranded DNA bacteriophage ΦX174. In one early paper, Hutchison and colleague Marshall Edgell demonstrated the 'salvage' of mutant phage by annealing mutant genomic DNA with enzymatically cleaved fragments from wild-type phage[1]; these findings suggested the possibility of DNA sequence modification, but without sequence data and access to targeted oligonucleotides of appropriate length, this technique's potential could not be realized.

A sabbatical in Fred Sanger's lab gave Hutchison the opportunity to participate in the completion of the first viral DNA sequence—that of ΦX174—using Sanger's 'plus-minus' technique[2]. Also on sabbatical with Sanger's group was Michael Smith, a former post-doc with H. Gobind Khorana, the nobel laureate chemist who pioneered oligonucleotide synthesis. Smith had subsequently moved to the University of British Columbia, where his group worked toward improved synthesis techniques.

The two investigators recognized the opportunity to develop a viable mutagenesis strategy, and saw in ΦX174 the ideal substrate. "We had mutations where we knew what the mutant and the wild-type sequence was," recalls Hutchison, "and we could design oligonucleotides to go either way, to go from the mutant back to the wild-type, or from the wild-type to the mutant, and we knew what the phenotypes would be... and beyond that, these were the only gene sequences there were!" Smith's team designed oligonucleotides to mutate the phage lysis gene; these were annealed to phage DNA, and polymerase was used to generate complete double-stranded molecules, which were transfected into *Escherichia coli*. Both wild-type and mutant virus particles resulted, with the mutant phenotypes matching those observed in lysis mutants that Hutchison and Edgell had previously generated by nonspecific mutagenesis, confirming the success of their strategy[3].

Smith's group went on to further develop this technique, while Hutchison and Edgell collaborated on alternate lines of genetic study. In 1993, Smith received the Nobel Prize in medicine for his research. Although the Nobel committee may have overlooked Hutchison's essential contributions to this work, he continued to make important strides in genetic research, including a strategy for large-scale, 'complete' mutagenesis, which his group notably applied to HIV-1 protease[4].

A major breakthrough for Hutchison and Smith's technique came in 1983, with the publication by Greg Winter and Alan Fersht of the first successful use of this method for the modification of an enzyme[5]. This laid the foundation for the field of protein engineering, now one of the most vital areas of biological research.

Fersht continued his work in protein engineering, and remains enthusiastic about this approach, but is equally enthusiastic about how much simpler it has become: "When we started doing these things, it was really quite horrendous... in those days, we had to make our own oligonucleotides manually, we had to purify our own polymerases, and the mutation efficiencies were about 1%. Nowadays, my students just go out and buy a kit!"

Modern mutagenesis owes much to the work of investigators such as Thomas Kunkel, who introduced a uracil-based selection scheme that raised the efficiency of heteroduplex-based mutagenesis to nearly 100% (ref. 6). More recently, several PCR based methods have emerged, like the 'megaprimer' approach[7,8] described in the following pages. Such techniques offer a rapid and budget-friendly alternative, and, as mutagenesis forefather Hutchison points out, "make [mutagenesis] more accessible to people who just... want one mutant, and they don't have to mess around with single-stranded DNA."

In light of today's unprecedented access to custom oligonucleotides and genomic sequence data, and the relative ease with which feats in protein engineering can be achieved, it may be worth remembering how much is owed to one humble bacteriophage.

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