Longer is better—random elongation mutagenesis

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The list of randomization methods for in vitro protein evolution just got longer. In this issue, Urabe and colleagues[1] present a new technique—random elongation mutagenesis—that exploits the stabilizing capacity in certain proteins of terminal arms of amino acids that fold across a susceptible part of the structure, stabilizing it under unfavorable conditions such as high temperature. By adding short peptide chains containing randomized sections to the carboxy terminus of *Bacillus stearothermophilus* catalase, these authors have succeeded in creating enzymes that demonstrate enhanced thermostability compared with the wild type.

The development of techniques for manipulating nucleic acids in vitro has coincided with equally rapid advances in our ability to determine the three-dimensional structures of proteins. This has provided the opportunity both to design novel enzymes by mutating the sequence in specific places and to find out a great deal about exactly how enzymatic catalysis works. Occasionally, the product of these efforts has had a sufficient advantage over the original wild type to justify its use in industrial applications.

Site-directed mutagenesis requires a full three-dimensional structure from X-ray crystallography, as well as all the tricks of the molecular biologist. Skeptics often argue that this process takes a perfectly good enzyme and converts it into a series of inferior ones. Nevertheless, one of the main aims is to produce enzymes that nature has not evolved, simply because they have not been needed. Although the whole procedure is now becoming increasingly easy to carry out, with high-resolution graphics and sophisticated molecular dynamics programs available, designed mutations unfortunately tend to misbehave as often as they do what was predicted of them.

A parallel procedure, which might be described as rogue science, has surpassed the classical inductive approach of site-directed mutation in its ability to achieve desired novel properties. Random mutagenesis, also known as directed evolution, takes a leaf out of Nature's book and is now a well-established procedure (see Fig. 1). It involves the generation of thousands of random mutants and their subsequent screening to identify variants that may have the new desired properties. This turns the traditional approach on its head; instead of finding out everything possible about a structure, and then trying to predict what mutation ought to give some desired effect, the random method assumes nothing about the structure, but automatically selects a mutant that does work. (Later, one can find out what happened and try to explain it!)

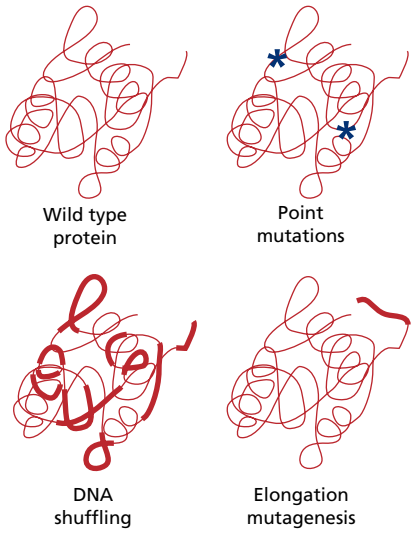

Figure 1. Three different approaches for molecular evolution of a protein.

Some of the first successful demonstrations of the random mutation approach were in the laboratory of Frances Arnold[2], who continues to be a major force in the area[3], but several other groups have made substantial contributions to the development of new methods. Most random mutagenesis work now uses the polymerase chain reaction (PCR), in conditions where it makes an occasional mistake, generating a library of mutant genes. More focused random procedures use mixed synthetic oligonucleotides inserted into the gene in place of the natural sequence to produce many mutants concentrated around a target site[4].

The DNA shuffling[5,6] method pioneered by Pim Stemmer also uses PCR to copy Nature, essentially recreating natural recombination in vitro (see Fig. 1). This approach has recently been extended to recombine homologous genes from different species[7]. Many of these methods have been used with the target of increasing enzyme stability, a property that at our current stage of understanding is particularly difficult to improve by design, but which is particularly desirable for industrial processes.

In the present paper, Urabe and colleagues[1] now stretch the possibilities still further with the technique of random elongation mutagenesis (see Fig. 1). The methodology is simple; requiring only the design of suitable ligation systems to insert a partially randomized oligonucleotide ahead of the stop codon in order to introduce short chains—16 amino acids in length—into the carboxy terminus of a protein.

The authors isolated *Escherichia coli* clones expressing active catalase and then studied their properties. Of 58 clones selected, 27% had higher thermostability than the wild type (which was already very thermostable, as it comes from a thermophilic organism). This is a much higher proportion of positives than alternative randomized methods have ever achieved.

Moreover, when the same approach was used with a mutant version of the catalase that was initially less thermostable, nearly all of the elongated variants were more stable than the starting material. This particular mutant, generated by what we might already call "traditional" random mutagenesis, had previously been selected for its better peroxidase (relative to catalase) activity, compared with the wild type[8]. But in creating these altered catalytic properties, the thermostability had been compromised. A combination of the two procedures—random point mutations to change substrate specificity, followed by elongation mutagenesis to increase thermostability—allowed isolation of a variant with both desired properties.

Random elongation mutagenesis is unlikely to be useful for changing catalytic properties, but as demonstrated in this example, it can be very successful in stabilizing an enzyme without compromising its enzymatic ability. Of course, catalase is an enzyme that does not need to undergo large conformational changes during catalysis. The method may not work so well with enzymes that do need more flexibility. But the main idea is attractive, and we can look forward to seeing it developed further, adding shorter or longer chains, at either end, both ends, or even in the middle of an existing protein molecule to stabilize it.

We are already familiar with adding bits to our proteins in order to purify or detect them easily. Perhaps one day we will have a "universal fusion" peptide that will cause overexpression, stabilize, and enable purification and detection all at once?

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