

Science & Technology

● SYNTHETIC BIOLOGY

A bacterium that can read man-made DNA

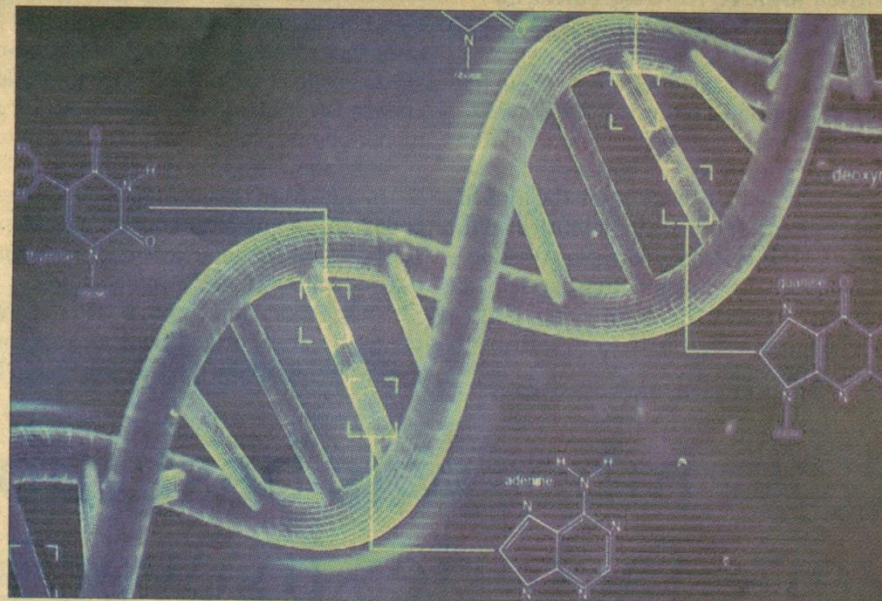
Biologists expand life's alphabet to include two new letters

ALL LIFE ON EARTH uses the same four chemical letters, known as bases, to store genetic information in the form of DNA. Three bases form a codon, a genetic "word" that represents one of 20 natural amino acids. A string of codons can be read by the machinery inside cells and turned into long chains of amino acids. These chains fold up into proteins, which carry out many of the innumerable jobs necessary for life.

Earlier this year Floyd Romesberg of the Scripps Research Institute in La Jolla, California, and his colleagues unveiled an engineered organism that does things rather differently. Their bacterium stores information using a six-letter genetic alphabet comprising the four usual bases (A, G, C and T, or adenine, guanine, cytosine and thymine) plus two artificial ones called NaM and TPT3.

In a paper published this week in *Nature*, Dr Romesberg and his colleagues go a step further, by describing how they have coaxed their bacterium into making proteins containing amino acids that are not found in nature. Each unnatural amino acid to be inserted is represented by a novel codon that includes one of the team's synthetic bases. In other words, their bacterium can quite happily read an entirely new, human-created extension to the standard genetic code, and use the instructions to produce proteins that no organism naturally makes. The hope is that one day this method could be used to make new drugs, polymers or catalysts.

To make their bug, the researchers had



to find molecules that could serve as their artificial bases. The four natural bases in DNA pair up in a specific way: guanine binds to cytosine and adenine to thymine. Double-stranded DNA is held together by the interactions between thousands of bases pairing up with their partners on the opposite strand. The binding rules mean that when the strands separate during cell division it is possible to construct new copies of the DNA using the existing strands as templates. The team screened thousands of molecules to find two that would pair up and be copied as faithfully as natural ones.

They then inserted into their bacterium a gene (made from the four standard bases) that encodes a transport protein (found in *Phaeodactylum tricornutum*, an alga), which allows the bacterium to ship the new bases across its cell wall. In earlier work, the scientists showed that their engineered

bug can incorporate the two artificial bases into its genome, and will happily copy DNA strands containing them when it reproduces.

Three more steps were necessary, however, before the bacterium could actually produce the new proteins encoded by its novel bases. To make proteins, cells first transcribe a piece of DNA into another long polymer called messenger RNA (mRNA). As its name suggests, this is the stuff that carries production instructions to the ribosomes, the cellular factories where proteins are assembled. The team thus needed to make mRNA versions of the two synthetic DNA bases.

Once messenger RNA arrives at the ribosome, yet another chemical, called transfer RNA (tRNA), gets involved. Its job is to carry the required amino acid to the ribosome and attach it in the correct place. At one end of this molecule is a triplet of bases

that allow it to recognise a particular codon. Its cargo is attached to the other. The cell's tRNAs had to be modified to recognise the novel codons. And the enzymes that load amino acids onto pieces of tRNA also needed tweaking, to be able to cope with the unnatural amino acids that are the ultimate point of the exercise.

To demonstrate that all this had worked as planned, the team instructed their bacterium to make a modified version of green fluorescent protein (GFP). That is a molecule found naturally in jellyfish, but which is now widely used to tag other molecules for study since, as its name suggests, it fluoresces under the right sort of light. In their first experiment, they showed that an unnatural codon (specifically A-NaM-C) could be used to insert a single molecule of serine, a natural amino acid, into GFP. In two further experiments they tried inserting first one, and then another, artificial amino acid into GFP. The artificial amino acids they used resembled natural ones but carried an additional chemical group, which allowed the researchers to identify them. In both cases, they found that more than 95% of the protein produced by the bacteria contained the synthetic building block in question.

As a next step Dr Romesberg hopes to extend the bacterium's genetic vocabulary. The two new bases mean 152 more codons are available to represent non-natural amino acids. Proteins made with synthetic ingredients should be more easily tailored to have desirable therapeutic properties (to be longer lasting, for example, or more powerful) than the natural sort. Synthorx, a biotech firm based in La Jolla which Dr Romesberg founded in 2014, was set up to explore exactly such possibilities.

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