WEIRD VIRAL DNA SPILLS SECRETS TO BIOLOGISTS

Bacterium-infecting viruses have specialized enzymes to make genes with an alternative nucleobase.

By Ewen Callaway

'Alien' genomes can be found on Earth. Some viruses that infect bacteria use an alternative genetic alphabet that's distinct from the code used by nearly all other organisms — and two teams have spelt out how the system works.

The studies show how dozens of these bacteriophages (or 'phages') write their genomes using a chemical base called 2-aminoadenine, Z for short, instead of adenine — the A in the A, T, C and G bases of genetics textbooks.

"Scientists have long dreamed of increasing the diversity of bases. Our work shows that nature has already come up with a way to do that," write Suwen Zhao, a computational biologist at ShanghaiTech University in China, and her team in a 29 April *Science* paper, showing how 'Z-DNA' is made[1]. Researchers in France described similar insights in a pair of papers in the same journal[2,3].

Bond booster

Scientists in the Soviet Union were the first to discover Z-DNA, in the late 1970s, in a phage called S-2L, which infects photosynthetic bacteria[4]. They found that the phage DNA behaved oddly when its two helical strands were melted apart. The bond that forms between G and C bases breaks at a higher temperature than does that joining A and T, and the phage's DNA behaved as if it was made mainly from G and C. But analysis showed that the phage had replaced A with Z, which forms a stronger bond with T.

"It looked like something transgressive," says Philippe Marlière, an inventor and geneticist at the University of Evry in France, who led one of the *Science* studies.

Follow-up studies showed that S-2L's hardier genome was resistant to DNA-chomping enzymes and other anti-phage defences that bacteria wield. But researchers didn't know how the Z-DNA system worked or whether it was common. Z-DNA is only one of a host of modifications known to exist in phage DNA.

To answer those questions, a team led by Marlière and Pierre-Alexandre Kaminski, a biochemist at the Pasteur Institute in Paris, sequenced the phage's genome in the early 2000s. They found a gene that's potentially involved in one step of making Z-DNA, but not in others. But the sequence had no matches in genomic databases at the time, and the quest to understand the basis of Z-DNA hit a dead end.

Marlière and his colleagues patented the S-2L genome, but also made it public, and Marlière continued to scour genomic databases. Finally, in 2015, the team got a hit: a phage that infects aquatic bacteria of the genus *Vibrio* harboured a gene that matched a stretch of S-2L's genome. The gene encoded an enzyme similar to one that bacteria use to make adenine. "It was an exhilarating moment," says Marlière.

In 2019, Zhao's team found similar database matches. Both teams showed that the phages all had a gene named PurZ. This codes for an enzyme involved in making the Z nucleotide. They then identified more enzymes — encoded in the genomes of bacteria that the phages infect — that complete the pathway.

But a key question lingered. The enzymes that the teams identified produced the raw ingredient for Z-DNA — a molecule called dZTP — but that didn't explain how phages insert the molecule into DNA strands, while excluding A bases (in the form of a chemical called dATP).

Here, the teams' conclusions differed. Alongside PurZ in the *Vibrio* phage's genome sits a gene that makes a polymerase enzyme, which copies DNA strands. Marlière and Kaminski found that the phage polymerase incorporates dZTP into DNA, while cutting out any A bases. "This explained to us why A was excluded," says Kaminski.

Zhao thinks this isn't the whole story. Her work suggests that another phage enzyme is needed to break up dATP but preserve dZTP. Her team found that increasing dZTP levels relative to those of dATP was enough to trick a cell's polymerase into making Z-DNA.

Missing links

It's still unclear how hosts keep Z out of their DNA or how cellular machinery that reads DNA to make proteins copes with Z-DNA. It's also not fully understood how Z-DNA is copied.

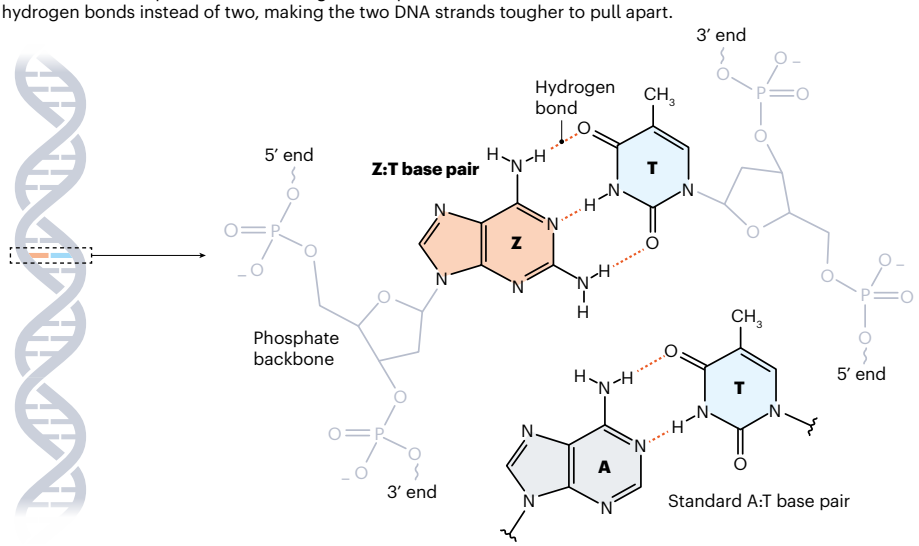
The functionality of host enzymes could be improved or impaired when working on Z-DNA, says David Dunlap, a biophysicist at Emory University in Atlanta, Georgia, who has found that an *Escherichia coli* enzyme struggles to coil and bend the exotic double helix[5]. The discovery of more phages with Z-DNA, and of genes involved in making it, should help researchers to understand how phages benefit from using it.

On the applications front, Z-DNA's hardiness could make the nascent technique of DNA data storage more stable and long-lasting. Nanomachines known as DNA origami might fold into shape faster when made of Z-DNA.

Steven Benner, a synthetic biologist and founder of the Foundation for Applied Molecular Evolution in Alachua, Florida, hopes that the new studies will rattle researchers into realizing the power of altering the genetic alphabet. "The fact that nature has taken a small step in the same direction may be the intellectual caffeine needed to get the molecular-biology community to understand that DNA can be improved, and beneficially so," says Benner.

HARDY GENOME

The 'Z' base that replaces the 'A' in the genetic alphabet of certain viruses forms three hydrogen bonds instead of two, making the two DNA strands tougher to pull apart.



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