MOLECULAR ENGINEERING

Double the fun

An eight-letter genetic alphabet can form a double helix resembling natural DNA and can be transcribed into RNA.

A boom in DNA technologies has provided researchers with a broad range of useful tools, including DNA high-throughput sequencing, long-read sequencing, and synthesis. These have been enabled by scientists' understanding of the structure of the double helix. However, a fundamental question remains: could functional DNA be made up of more than 4 bases? If so, what would DNA with additional bases enable researchers to do? Steven Benner, working at the Foundation for Applied Molecular Evolution in Florida, and colleagues demonstrate that a double helix containing two additional nucleobase pairs is stable and can be transcribed into a functional aptamer.

Since the double helix was first described in 1953, the two rules that were initially thought to underpin base pairing—that is, that large purines pair with small pyrimidines, and that hydrogen-bond donor bases pair with hydrogen acceptors—have been shown to be nonessential, at least to a certain extent. Scientists built DNA with nucleobase analogs adding a third pair, but if this additional pair lacked interbase hydrogen bonding, the double-helix structure was unstable. Benner's group had also shown that a third pair using hydrogen bonding would support a stable double helix that could be PCR-amplified, sequenced, and transcribed into six-letter RNA.

Could the limits of DNA architecture be pushed further? Seeking to engineer an eight-letter (thus named 'hachimoji') DNA alphabet, Benner and colleagues designed and synthesized 94 DNA oligonucleotide duplexes comprising the four standard nucleotides (A, T, G, C) plus two pyrimidine (Z, S) and two purine analogs (P, B). The diversity of sequences represented in these duplexes would allow a good estimation of how synthetic base pairs behave when flanked by a range of natural and analog bases. The thermodynamic data they obtained from assembling and melting the 94 duplexes showed that, as they had predicted, they could measure S:B and Z:P pairing in addition to A:T and G:C. And in high-resolution crystal structures of three hachimoji DNA sequences (16-mers), they observed a double helix with small differences in the pairing characteristics of the analogs, falling well in the range of natural DNA pairing.

To test the functionality of their new genetic alphabet, the scientists sought to show that it could be transcribed as RNA. They searched for T7 RNA polymerase variants that would be able to incorporate ribonucleotide analogs during the transcription of hachimoji DNA. Then, to show that such a transcript would be functional, they designed a hachimoji DNA sequence corresponding to a fluorescent RNA aptamer and transcribed it. The hachimoji aptamer folded properly and fluoresced.

The fact that the four-letter alphabet that underpins life on our planet can be doubled might signify that a range of DNA architectures could support life. More concretely, the increased information density of hachimoji DNA might prove useful in increased hybridization specificity, DNA storage systems, barcoding, and combinatorial tagging. It could also be used to select aptamers. "One advantage of this might be that it allows you to make aptamers built from more diverse things than just the four natural nucleotides and thus might have otherwise impossible properties or functions," says Floyd Romesberg, a professor at Scripps Research. In a living organism, "it could allow it to make proteins made from more than the 20 canonical amino acids for all sorts of applications," he adds. Although whether hachimoji DNA can be amplified or used in a cell has not yet been demonstrated, possibilities abound for the extra DNA building blocks.

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Published online: 28 March 2019
https://doi.org/10.1038/s41592-019-0377-z

Research papers
Hoshika, S. et al. Hachimoji DNA and RNA: a genetic system with eight building blocks. *Science* **363**, 884–887 (2019).