GENOMICS

Arrival of the Argonautes

An Argonaute protein enables precision genome editing in mammalian cells.

Genome editing applications using the RNA-guided DNA endonuclease Cas9 are now widespread, but the enzyme has limitations. Chunyu Han and colleagues at Hebei University of Science and Technology sought an endonuclease that is less tolerant of mismatches between guide and target and that does not have the same stringent requirements for guide design. They set their sights on Argonaute proteins, endonucleases that typically target RNA, although some can cleave DNA that matches a single-stranded DNA (ssDNA) guide.

Previously described Argonaute DNA endonucleases work only at high temperatures (>65 °C), but the authors found that NgAgo, an Argonaute from *Natronobacterium gregoryi,* can cleave DNA *in vitro* at 37 °C when pre-loaded with ssDNA guides. The guide itself simply needed to be 5′-phosphorylated and to consist of 13–25 nucleotides (nt). When NgAgo was expressed in human 293T cells, it bound cotransfected 5′-phosphorylated ssDNA guides. Importantly, it did not seem to bind endogenous DNA, minimizing the likelihood of off-target binding. Copurified NgAgo and ssDNA guides led to consistent cleavage of plasmid DNA *in vitro*. Unlike Cas9, NgAgo seemed to randomly remove 1–20 nt from the target region after cleavage.

The authors then tested NgAgo on endogenous loci in cells. Five different 24-nt ssDNA guides were used to target exon 11 of the gene *DYRK1A*, and each one led to cleavage. Further testing using 47 guides targeting eight other genes showed that NgAgo could consistently cleave target sequences with high efficiency. To test specificity, the researchers introduced single-nucleotide mismatches at each position of the 24-nt guide; each one reduced cleavage efficiency by 73–100%. Three mismatched nucleotides in a row completely prevented cleavage.

Side-by-side comparisons showed that NgAgo was able to edit *DYRK1A* with similar efficiency as Cas9. Moreover, NgAgo does not require a PAM motif and was more effective in cleaving a GC-rich target, for which Cas9 requires a guide RNA prone to secondary structure.

Although further characterization is required, the low tolerance of mismatches suggests that NgAgo-based genome editing is unlikely to be prone to off-target effects. Moreover, the simplicity of the guide design offers another potential advantage of NgAgo over Cas9 for genome editing applications.

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RESEARCH PAPERS

Gao, F. *et al.* DNA-guided genome editing using the *Natronobacterium gregoryi* Argonaute. *Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3547 (2016).