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News & Views

NgAgo: an exciting new tool for genome editing

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Site-directed DNA endonucleases are powerful tools for genome editing. When introduced into cells, these systems can bind to a target DNA sequence in the genome and create a DNA double-strand break (DSB), the repair of which leads to varied DNA sequence modifications. The initial efforts on developing these tools were focused on engineering homing endonucleases [1] and zinc finger nucleases (ZFN) [2, 3], then transcription activator-like effector nucleases (TALENs) [4–6]. All these systems rely on designing protein module to recognize specific DNA sequences. Emerged from the acquired immune system of bacteria and archaea, clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated proteins (Cas) system is an RNA-guided DNA endonuclease system in which Cas9 endonuclease is directed to specific genomic locus by a guide RNA that is complementary to target DNA sequence [7, 8]. Due to the simplicity of RNA-guided nature and robustness of the system, CRISPR-Cas9 rapidly became the method of choice for genome editing since 2012.

In a recent issue of *Nature Biotechnology*, research team led by Dr. Chunyu Han developed a novel genome editing system, based on *Natronobacterium gregoryi* Argonaute protein [9]. RNA interference (RNAi) plays important roles in gene regulation in eukaryotes. In the siRNA pathway, the target strand of siRNA is loaded into the RNA-induced silencing complex (RISC) and guides the complex to cleave the target mRNA with perfect complementarity. In both plants and animals, the catalytic core of RISC is Ago2, which catalyzes the mRNA cleavage. Ago2 and the Argonaute family members contain four domains: N, PAZ, MIDI, and PIWI. There are a large number of Argonaute orthologs in prokaryotes sharing very similar structure with mammalian Argonaute [10]. Although the RNAi pathways have been well characterized in eukaryotes, little is known about the functions of prokaryotes Argonaute proteins. Several studies elucidated the crystal structures of prokaryotes Argonaute proteins, serving as models for the understanding of the human counterparts [11–15]. These studies also demonstrated in vitro that TtAgo and AaAgo could catalyze targeted cleavage of single-stranded (ss) DNA and RNA using ssDNA oligo as guide [11, 14]. Furthermore, a recent study demonstrated that TtAgo could degrade DNA plasmids in vitro and in bacteria, using ssDNA as the guide [16]. However, TtAgo is derived from *T. thermophiles*, which lives in high-temperature (85 °C) habitats, and the targeted degradation of plasmid is only efficient at 65 °C or higher temperatures [16].

Dr. Han's team identified NgAgo as an Argonaute protein that is functional at 37 °C. They demonstrated that purified Argonaute protein cleaved plasmids in vitro, when complexed with single-stranded guide DNA (gDNA) with 5′ phosphorylation. They further showed that when the plasmid encoding NgAgo and gDNA was transfected into human 293T cells, NgAgo specifically cleaved eGFP plasmid, leading to a significant reduction in eGFP protein in the cells. Using this platform, they defined the optimal gDNA length as 24 nt. More importantly, they showed that NgAgo with nuclear localization signal (NLS) efficiently generated mutations at endogenous genomic loci in human genome, with an efficiency comparable or higher than the well-established CRISPR-Cas9 system.

NgAgo system has several very interesting characteristics; here I discuss their potential pros and cons:

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1. NgAgo uses 24 nt ssDNA with 5′ phosphorylation as guide. As ssDNA oligos can be readily synthesized industrially, there is no need for any molecular cloning to direct NgAgo to a specific target site, and one can simply order the guide DNA oligo from company and co-transfect it with NgAgo plasmid into cells. It could not be easier! However, given that gDNA cannot be simply provided using a viral vector, efficient delivering of both NgAgo-expressing vector and gDNA into tissue or organ could be challenging. Also, although the easy synthesis of oligos in high-throughput manner confers this system obvious advantage for loss-of-function genetic screening in an arrayed format, as the gDNA currently cannot be synthesized from vector, it is not feasible to do screening in a pooled format.
2. Short time window for NgAgo to complex with gDNA. As the time window for NgAgo to complex with gDNA after it gets expressed in human cells is short, it might need optimization of transfection protocol for each cell type to get the system work efficiently. It is very curious why this is the case, and how the translation of NgAgo protein and loading gDNA are coupled together. By better understanding the mechanisms and kinetics of these events, we will be able to improve the NgAgo performance significantly.
3. NgAgo works efficiently in high GC target site and has no PAM restriction. This is likely a significant advantage over CRISPR-Cas9 system. However, while the published results are very exciting, the general applicability remains to be tested in more genomic loci and more cell types other than the 293T cells. While Dr. Han showed that NgAgo had relatively low tolerance for gDNA-target mismatch, the specificity of NgAgo system needs more systematic characterization. Last, as the 5′ phosphorylated ssDNAs might be identified as dangerous invading nucleic acid species, whether the use of ssDNAs could induce innate immunity and other cellular responses becomes an important issue.

Despite all these uncertainties, NgAgo system will likely be further improved by protein engineering such as directed evolution, or rational design based on protein crystal structure. More importantly, Ago proteins are present in various prokaryote species, and many new enzymes are waiting to be discovered as better tools for genome editing.

Conflict of interest The author declares that there is no conflict of interest.

References

1. Silva G, Poirot L, Galetto R et al (2011) Meganucleases and other tools for targeted genome engineering: perspectives and challenges for gene therapy. Curr Gene Ther 11:11–27
2. Urnov FD, Miller JC, Lee YL et al (2005) Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature 435:646–651
3. Urnov FD, Rebar EJ, Holmes MC et al (2010) Genome editing with engineered zinc finger nucleases. Nat Rev Genet 11:636–646
4. Boch J, Scholze H, Schornack S et al (2009) Breaking the code of DNA binding specificity of TAL-type III effectors. Science 326:1509–1512
5. Bogdanove AJ, Voytas DF (2011) TAL effectors: customizable proteins for DNA targeting. Science 333:1843–1846
6. Moscou MJ, Bogdanove AJ (2009) A simple cipher governs DNA recognition by TAL effectors. Science 326:1501
7. Doudna JA, Charpentier E (2014) Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346:1258096
8. Hsu PD, Lander ES, Zhang F (2014) Development and applications of CRISPR-Cas9 for genome engineering. Cell 157:1262–1278
9. Gao F, Shen XZ, Jiang F et al (2016) DNA-guided genome editing using the Natronobacterium gregoryi Argonaute. Nat Biotechnol. doi:10.1038/nbt.3547
10. Makarova KS, Wolf YI, van der Oost J et al (2009) Prokaryotic homologs of Argonaute proteins are predicted to function as key components of a novel system of defense against mobile genetic elements. Biol Direct 4:29
11. Yuan YR, Pei Y, Ma JB et al (2005) Crystal structure of *A. aeolicus argonaute*, a site-specific DNA-guided endoribonuclease, provides insights into RISC-mediated mRNA cleavage. Mol Cell 19:405–419
12. Ma JB, Yuan YR, Meister G et al (2005) Structural basis for 5′-end-specific recognition of guide RNA by the *A. fulgidus* Piwi protein. Nature 434:666–670
13. Wang Y, Juranek S, Li H et al (2008) Structure of an argonaute silencing complex with a seed-containing guide DNA and target RNA duplex. Nature 456:921–926
14. Wang Y, Juranek S, Li H et al (2009) Nucleation, propagation and cleavage of target RNAs in Ago silencing complexes. Nature 461:754–761
15. Wang Y, Sheng G, Juranek S et al (2008) Structure of the guide-strand-containing argonaute silencing complex. Nature 456:209–213
16. Swarts DC, Jore MM, Westra ER et al (2014) DNA-guided DNA interference by a prokaryotic Argonaute. Nature 507:258–261