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PERSPECTIVES

An NgAgo tool for genome editing: did CRISPR/Cas9 just find a competitor?

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Abstract While CRISPR/Cas9-mediated genome editing technology has been experiencing a rapid transformation during the past few years, a recent report on NgAgo-mediated single-stranded DNA-guided genome editing may offer an attractive alternative for genome manipulation. While it's too early to predict whether NgAgo will be able to compete with or be superior to CRISPR/Cas9, the scientific community is anxiously waiting for further optimization and broader applications of the NgAgo genome editing technology.

With the rapid progress being made in genome sciences, effective genome engineering holds great promise both in understanding the molecular bases of human diseases and in treating human disorders with identifiable alterations in the genome. Since the advent of the effective but clumsy zinc-finger nuclease-based TALEN (transcription activator-like effector nucleases) technology,[1–3] we have been searching for simple and effective approaches for genome manipulation. The past few years have witnessed a rapid rise of the RNA-guided CRISPR/Cas9 technology from obscurity.[4–8] CRISPRs (Clustered Regularly-Interspaced Short Palindromic Repeats) are segments of prokaryotic DNA containing short sequence repetitions, while bacterial endonuclease Cas9 can be programed by a small guide RNA (gRNA) to induce a double-strand break at a desired genomic locus that is followed by a protospacer-adjacent motif (PAM).[9] The resulting double-strand break can either be repaired by homology-directed repair (HDR) or by non-homologous end joining (NHEJ), which is usually imprecise, resulting in small insertions or deletions (indels).[4,7] This technology has been widely used to rapidly generate gene knockouts in various organisms. Significant efforts are being devoted to optimizing the current CRISPR/Cas9 system and/or to identifying more Cas9-like nucleases with better efficiency and specificity.[7,10–12]

However, a recent report might have changed the landscape of genome editing technologies.[13] Gao F., et al identified a member of the Argonaute endonuclease family, called *Natronobacterium gregoryi* Argonaute (NgAgo), which was shown to effectively carry out DNA-guided genome editing in mammalian cells.[13] The Argonautes are a family of endonucleases that use 5′-phosphorylated short single-stranded nucleic acids as guides to cleave targets.[14,15] Argonaute proteins play an important role in RNA interference and microRNA processing.[14,16] Several Argonautes isolated from thermophiles can use single-stranded (ss) DNA guides to cleave DNA targets at high temperature.[17–19] In their study, Gao F., et al demonstrated that the NgAgo endonuclease was able to bind 5′-phosphorylated single-stranded guide DNA (gDNA) of ~24 nucleotides and efficiently generate gDNA sequence-specific DNA double-stranded breaks at 37 °C. This editing process did not require the presence of PAM. NgAgo was shown to remove several nucleotides in the target region, although it seemingly does not have any exonuclease activity.[13] By assessing the ability of 47 different ssDNA guides targeting eight genes in the human genome, the authors reported 21–41% targeting efficiencies among all guides tested. The authors subsequently demonstrated that the NgAgo–gDNA system exhibited a low

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tolerance to guide—target mismatches, as mismatches between the ssDNA guide and target reduced the efficiency of cleavage. A comprehensive analysis of single mismatches at all 24 positions revealed that all positions were important, with positions 8—11 being particularly important, and that the presence of three consecutive mismatches eliminated all activity.[13] The authors further showed that NgAgo, unlike CRISPR/Cas9, was highly efficient in editing almost any sequence, including G/C-rich genomic sequences.[13]

While still at an early development stage, NgAgo has the following distinctions and potential advantages over the CRISPR/Cas9 system: (1) NgAgo utilizes 5′-phosphorylated ssDNA guides (~24 bp) to cleave supercoiled DNA; (2) mammalian cells have very low levels of 5′P-ssDNA, eliminating the possible presence of off-target guides for NgAgo; (3) NgAgo is seemingly more specific than Cas9, as it completely loses activity when there are three mismatches; (4) NgAgo was about as effective as, if not more effective than Cas9 for DNA cleavage in mammalian cells; (5) NgAgo is a smaller protein, which is easier to express; and (6) NgAgo does not require a PAM and can target GC-rich genomic loci with high efficiency. Nonetheless, much more needs to be learned about the NgAgo system before any conclusions can be drawn about its potential applications. While the use of 5′-phosphorylated ssDNA as a guide provides a simple and convenient means for *in vitro* cell-based genome editing, this may limit NgAgo's utility for *in vivo* genome engineering unless approaches for efficient and targeted delivery of ssDNA guides are devised. It remains to be fully investigated whether NgAgo can effectively mediate homology-directed recombination with similar or better efficiency compared with CRISPR/Cas9. Structure-function studies of NgAgo may further optimize its genome editing activity. It's conceivable that more NgAgo-like proteins will be found in future studies.

For much of the scientific community however it is not important to determine whether NgAgo will out-compete CRISPR/Cas9, or vice versa. We are thrilled to have another cool tool in the genome engineering toolkit.

Conflict of interest

The authors declare no conflicts of interest.

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