NgAgo: a new genome editor

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Since the last decade, the field of genome engineering and functional genomics has been revolutionized at rapid speed with the development of sequence-specific nucleases (SSNs), such as zinc finger nucleases (ZFN)[1], transcription activator-like effector nuclease (TALEN)[2], and clustered regularly interspaced spacer palindromic repeats (CRISPRs)[3,4]. SSNs are designed to catalyse the targeted double-strand breaks (DSBs) at specific loci in the genome with unprecedented ease. These DSBs can be repaired by either non-homologous end-joining (NHEJ) pathway or homologous recombination (HR) pathway which ligates the breaks, resulting in small insertion, deletion or replacement. The NHEJ pathway is inherently error-prone but more frequent than the HR pathway which often produces random mutations. In contrast, the HR pathway mainly operates during S and G2 phase of cell cycle when sister chromatids are available as template. The SSNs (specifically ZFN and TALEN) are chimeric nucleases composed of two components: a designed DNA binding domain and a cleavage domain (Fok1), functioning as dimers. These SSNs have been used in a diverse range of organisms, but their utility is hampered by the requirement of sophisticated and costly design of individual proteins for each new target sequence and their undesired off-target effects. Moreover, TALENs are huge protein and so it becomes challenging to deliver them into specific cells or tissues[5]. Therefore, a more simplified and effective approach, CRISPR/Cas9 technology, was introduced with great expectations.

The soaring popularity of RNA-guided DNA endonuclease CRISPR/Cas9 technology has revolutionized the landscape of genome engineering. It allows researchers to selectively edit genome parts and replace them with new DNA stretches. The system is composed of a synthetic guide RNA (gRNA) and an endonuclease Cas9 of type-II CRISPR. Further, gRNA consists of two small non-coding RNAs: CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA)[6]. gRNA directs Cas9 to the target region that is complementary to the gRNA sequence near a 5′–NGG–3′ sequence also called protospacer adjacent motif (PAM). gRNA binds to the complementary target DNA via Watson–Crick base pairing. Cas9 recognizes the PAM sequence which leads to the activation of RuvC and HNH domain of Cas9 that induce a DSB at the chosen site in the target genome[7]. In addition to genome editing, CRISPR/Cas9 technology can also be used to target transcriptional modulation, epigenome editing, mark genomic loci in living cells and human therapeutics applications by mutating the nuclease domains of Cas9 (called deactivated or dead Cas9; dCas9), which loses its catalytic activity but is still able to bind to the target site. Therefore, dCas9 in conjugation with gRNA functions as a synthetic transcription regulator. The CRISPR/dCas9-mediated gene expression, repression and activation are commonly called CRISPRi and CRISPRa respectively[8,9]. But off-target effect of Cas9 limits its wider and safer applicability.

NgAgo (*Natronobacterium gregoryi* Argonaute) is an Argonaute (Ago) protein derived from halo-alkaliphilic archaea *N. gregoryi*. It can be used to cleave targeted DNA sequences and is the most recent addition in the arsenal of sequence-specific nucleases. Basically, Argonaute is a signature protein of RNA-inteference (RNAi) mechanism in eukaryotes that plays a central role in microRNA processing and gene regulation[10]. Recently, a study has revealed that NgAgo is able to cleave the target DNA at physiological temperature ~37°C when supplied with 5′-phosphorylated single-stranded DNA (ssDNA) of 24 nt length gDNA[11]. Previously, it has been shown that Argonaute isolated from *Thermus thermophilus* (TtAgo) and *Pyrococcus furiosus* (PfAgo) cleaves the target DNA at higher temperatures, i.e. >65°C (refs 12, 13). Gao *et al.*[11] showed that NgAgo only cuts with ssDNA and not with ssRNA nor ssDNA lacking 5′-phosphorylation (Figure 1). Unlike CRISPR/Cas9, it does not require any PAM sequence to cleave the target. The authors subsequently demonstrated that once gDNA is loaded onto NgAgo, it cannot exchange with another unbound ssDNA at 37°C. NgAgo has low tolerance of guide–target mismatch; if there is a single base mismatch it could reduce cleavage efficiency by 73–100% and completely suppress its cleavage activity by three consecutive mismatches, so there will be no/very low possibility of off-targets. On the other hand, Cas9 can tolerate five mismatches so there is possibility of cleave the undesired sequence also. Hence, NaAgo could be used for safer genome editing with higher specificity.

Gao *et al.*[11] performed a comparative analysis of editing efficiency of gRNA-directed Cas9 and gDNA-directed NgAgo system by targeting *DYRK1A* gene in HeLa cells. They found that the target cleavages by both the endonucleases are comparable. They also demonstrated that NgAgo endonuclease cleaves GC-rich target region more efficiently than Cas9. NgAgo can be potentially easily delivered into the target cell due to its small size (887 amino acids) and short gDNA by delivery vehicles such as viral vectors.

NgAgo represents another exciting genome engineering tool with several advantages when compared to CRISPR/Cas9 system: (1) It uses a 5′-phosphorylated gDNA for targeting which is much smaller and more stable than gRNA. (2) There are no barriers for target sequence

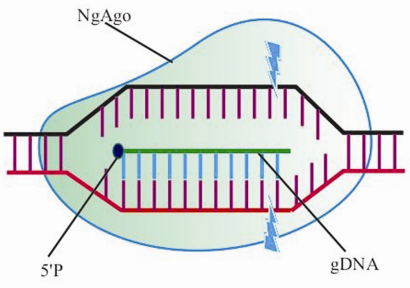

Figure 1. The NgAgo genome editor. The NgAgo nuclease is targeted by 5′-phosphorylated single-stranded gDNA to its target. NgAgo induces the double-strand breaks (DSB) in the target DNA by unknown nuclease domain. The DSB is repaired by either imprecise NHEJ pathway which leads to small indels, or HR pathway in the presence of double-stranded repair template DNA which leads to gene addition.

recognition as in the case of Cas9 which requires PAM sequence. (3) NgAgo is highly sensitive to mismatch and more specific than Cas9.

Now, the most relevant question arises: 'Will this new endonuclease NaAgo challenge the prevailing CRISPR/Cas9 system in the arena of genome editing?' According to us, this technology is still at its infancy and has a long way to go before it is fully accepted. There are many questions that remain to be answered for further improvement. (1) How does NgAgo bind to the target DNA and cleave both the strands? (2) How does it identify catalytic domains responsible for cleavage? (3) How does it remove several nucleotides at the cleavage site when it cuts the DNA. Despite these questions, the NgAgo system will likely become the central area of future biological research and development.

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