Molecular Therapy
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Commentary

A route to engineer a genome editor for gene therapy

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In the rapidly evolving field of genome editing, CRISPR-Cas system-based nucleases, base editors, transposases, and prime editors have emerged as versatile tools with substantial therapeutic potential.[1] However, a significant challenge lies in delivering these large tools *in vivo* to target tissues via adeno-associated viruses (AAVs), as their limited packaging capacity poses a major obstacle.[2] This is especially true for the promising cytosine or adenine base editors, which are composed of deaminases and Cas proteins.[3,4] To overcome this challenge, four strategies are often employed to minimize base editors: identifying homologs with smaller sizes, minimizing deaminases and/or Cas proteins or even promoters by deleting non-functional domains and amino acids, optimizing the architecture of base editors using SunTag or other recruiting systems to recruit deaminases to the target sites, and using a split strategy to express the functional components independently (Figure 1).

Among these strategies, identifying smaller variants of Cas proteins with high editing efficiency is a crucial solution for *in vivo* gene therapy. Although miniature Cas proteins such as Cas12f and the Tbpn system have been discovered,[5] the editing efficiency and scope of these Cas protein-mediated base editors are currently relatively limited. Thus, Cas9 homologs remain a promising choice for *in vivo* therapy due to their broad scope, high efficiency, strict targeting, and mature optimization pathway. The compact and adaptable CjCas9, derived from *Campylobacter jejuni*, is a strong candidate for AAV delivery, yet its editing efficiency in human cells needs to be engineered.[6,7] Enhancing the editing capability of CjCas9 *in vivo* is not only an incremental improvement but also a critical step forward in the field of gene therapy.

In this issue, Chen et al. have employed structure-guided protein engineering to significantly boost the limited editing efficiency of CjCas9.[8] By strengthening the interaction between CjCas9 and the phosphate backbone of the target DNA, they have successfully increased its DNA binding affinity. Through meticulous design and rigorous testing of multiple CjCas9 variants, the researchers have identified LDE-CjCas9 as the variant with the highest activity, which exhibits significantly improved performance in DNA binding, transcriptional activation, and base editing efficiency. This demonstrates that structure-guided amino acid substitutions offer a solid methodology for Cas protein improvement.

This study also offers comparative insights on the improved LDE-CjCas9 and other engineered CjCas9 variants, such as enCjCas9, evoCjCas9, UltraCjCas9, and SauriCas9. These variants incorporate specific mutations that expand the protospacer pdjacent motif (PAM) range or stabilize sgRNA interactions, illustrating diverse engineering strategies within the CRISPR toolkit. The head-to-head comparison of these variants performed by Chen et al. using the same structural design and guide RNAs evaluated the delivery flexibility and performance of LDE-CjCas9 in DNA cleavage, activation, and base editing. LDE-CjCas9 emerges as highly active in most base editing targets, and Chen et al. observed diverse efficiencies of CjCas9 variants among different targets in transcription activation. This is partially due to the possibility that variants obtained through different engineering methods may have different sequence preferences, highlighting the importance of selecting or screening the most suitable CjCas9 variant for a specific target. Specially engineered editing tools for specific uses might be a novel approach for gene therapy in clinic, although a universal tool is eagerly anticipated in this field.

The potential therapeutic application of LDE-CjCas9 is accentuated in the context of age-related macular degeneration (AMD), a chronic degenerative eye disease closely associated with the VEGFA-VEGFAR2 pathway. The study reveals that single-AAV-mediated delivery of LDE-Cj adenine base editor (ABE) efficiently induces base editing in the ocular retina of mice, exhibiting a higher editing efficiency than wild-type (WT)-CjABE at the Vegfr2 target. This not only illustrates the potential therapeutic application of LDE-CjABE in the mouse retina but also highlights the promise of AAV-mediated gene editing as a viable treatment strategy.

In conclusion, Chen et al. successfully generated a compact, efficient, and specific LDE-CjCas9 for mammalian genome engineering, demonstrating its advantages in base editing and transcriptional activation. This makes it a promising tool for further *in vivo* applications. Most importantly, it provides a general route to engineer genome editing tools using a structure-guided strategy. LDE-CjCas9 might not be the best version for *in vivo* gene therapy, but it paves an exquisite path to optimizing a small Cas9 homolog, which can be expanded to genome editing systems, highlighting the potential to unlock new frontiers in therapeutic genome editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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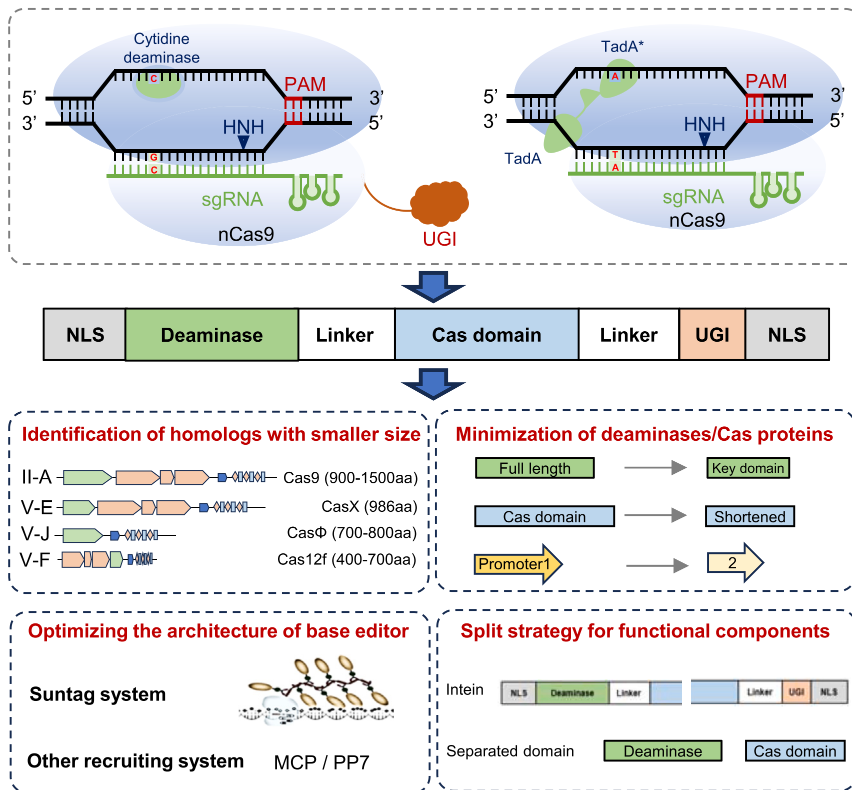

Figure 1. General strategy for minimizing base editors

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