Fundamental Research 4 (2024) 699–700

Contents lists available at ScienceDirect

Fundamental Research

journal homepage: http://www.keaipublishing.com/en/journals/fundamental-research/

Commentary

A commentary of "Novel approaches in precise genome manipulations from single nucleotides to large DNA segments": Top 10 Scientific Advances of 2023, China

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The life sciences field has experienced profound impacts from genome editing, a technology that enables precise genetic sequence modifications in living cells. Developments in genome editing have significantly propelled advances in biomedicine and agricultural breeding. With the introduction of CRISPR as a genome editing tool in 2012 [1], rapid progress has paved the way for emerging technologies such as base editing and prime editing [2,3]. These sophisticated editing methods facilitate more accurate genome modifications and promise to profoundly impact the future of precision medicine, crop improvement, and even the *de novo* design of organisms.

Recently, the scientific achievement entitled 'Novel approaches enable precise genome manipulation from single nucleotide to large DNA segments' was recognized as one of the Top 10 Scientific Advances, 2023. This breakthrough was completed by a team led by Prof. Caixia Gao from the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, and Dr. Kevin Tianmeng Zhao from Qi Biodesign in Beijing; these significant advancements propel the development and optimization of genome editing technologies (Fig. 1).

Base editors are precise genome editing technologies capable of performing specific single nucleotide substitutions with minimal bystander effects. A critical component of base editors is the deaminase enzyme, which is primarily responsible for performing a deamination reaction altering the identity of a DNA nucleotide. However, traditional sequence-based mining approaches over the past eight years have identified few single-strand DNA cytidine deaminases that all originate from only one protein family, thereby limiting the versatility of base editors.

To identify new deaminases for base editing, the team utilized artificial intelligence (AI)-assisted massively parallel prediction and characterization of protein structures, uncovering new relationships within the deaminase superfamily. This method led to the discovery of novel deaminases, enabling the development of a new suite of genome editors [4]. The work made three significant contributions: pioneering a new enzyme discovery method, tackling the delivery challenges of base editors using single adeno-associated virus (AAV) vectors, and addressing the inefficiency of base editors in soybeans.

Building upon their deaminase research, the team developed 'CyDENT', a novel base editing modality utilizing their newly discovered deaminase. Independently patented and owned by the team in China, CyDENT facilitates precise DNA base editing across mitochondrial, chloroplast, and nuclear genomes [5]. This achievement highlights the transformative effects of integrating AI with genome editing research, signaling a promising direction for future biotechnological advancements.

The continuous enhancement of genome editing tools has simplified the process of achieving gene knockouts, base mutations, and the insertion or deletion of small DNA fragments. However, challenges persist in precisely manipulating large DNA fragments exceeding kilobases in size. Given the critical roles of DNA fragment repetitions, deletions, inversions, and translocations in biological systems, the development of precise editing techniques for large DNA fragments holds significant importance in genome editing.

The team achieved efficient site-specific insertion of DNA fragments up to 10 kb in length in plants by combining the prime editing system with recombinases. Demonstrating this technique, they precisely inserted a 4.9 kb disease-resistant gene expression cassette into a genomic safe harbor, advancing the development of new rice germplasms resistant to rice blast disease. The editing system was named PrimeRoot and has overcome the technical bottleneck of manipulating large DNA fragments in plant genomes, laying the foundation for future crop breeding and plant synthetic biology efforts leveraging gene stacking [6].

Beyond technological innovations, this scientific progress has showcased the capability to fine-tune crop traits by systematically modifying the protein translation regulatory machinery using genome editing tools like base and prime editors. Upstream open reading frames (uORFs) are key cis-regulatory elements in eukaryotes and are known to repress gene translation. The team developed an efficient method to enhance protein expression by eliminating endogenous uORFs, leading to the creation of lettuce germplasm with increased vitamin C content and strawberries with progressively higher sweetness levels [7,8].

Building on their previous findings, the team subsequently developed precise genome editing strategies to downregulate gene translation by introducing new uORFs or extending existing ones to boost their inhibitory effect [9]. They combined these strategies to progressively downregulate gene translation, thereby creating a series of uORFs with varying inhibitory capacities. This nuanced control of protein translation in rice resulted in gradual phenotypic changes, such as in lamina

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https://doi.org/10.1016/j.fmre.2024.04.006

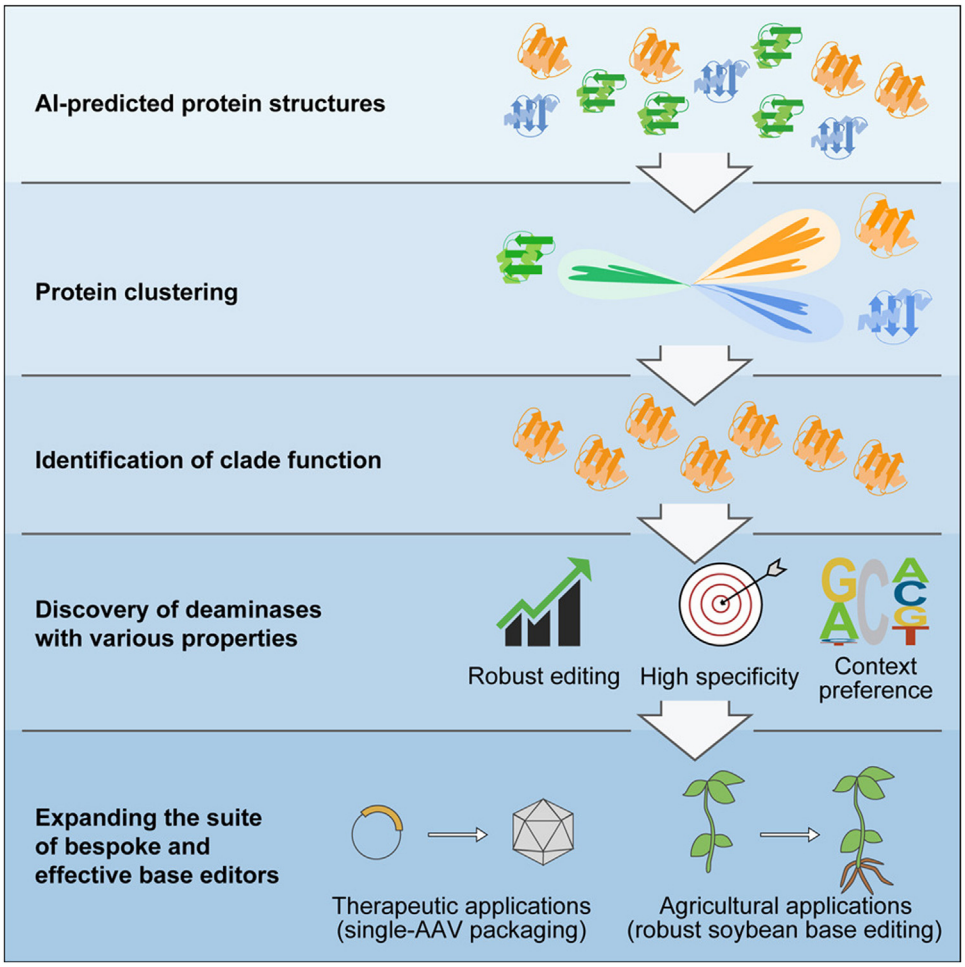

Fig. 1. Discovery of deaminase functions by structure-based protein clustering [4].

joint angle, plant height, and tiller number. Through strategic uORF manipulation, the team established an effective and widely applicable method for precise downregulation of gene translation in plants, promising significant improvements in crop breeding.

In conclusion, the series of research achievements stemming from this advancement has had a pioneering impact on technological innovation internationally. These discoveries are set to propel the ongoing progress in genome editing, enhancing its application across various biological domains [10].

Declaration of competing interest

The author declares that she has no conflicts of interest in this work.

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