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CRISPR Adventures in China

Caixia Gao[1,2] and Jia Chen[3,4]

Over the past two decades, developments in next-generation sequencing technologies have led a flourishing era of ''genome reading''. Recent CRISPR-based editing technologies are the beginning of a ''genome editing'' renaissance. CRISPR sequences were first discovered by microbiologists, but since 2012–2013, scientists across numerous fields worldwide have been attracted by the potential of CRISPR-Cas editing technologies as a versatile and accessible genome editing tool. A growing genome editing toolbox based on CRISPR systems involving Cas9, Cas12, Cas13, base editors (BEs), and prime editors (PEs) is advancing research in agriculture, biology, biotechnology, and medicine.[1–3]

China has established itself as one of leading nations in the CRISPR revolution due to the immense scientific curiosity of the research community and generous support from the Chinese government, including heavy investment from central, provincial, and city governments in China. Both the National Natural Science Foundation of the central government and the Ministry of Science and Technology have approved multiple projects in various disciplines. In September 2017, China launched the Committee of Genome Editing, Genetics Society of China, which became a platform for accessible communication and cooperation between scientists to accelerate the development of CRISPR research and applications in China. At this annual workshop, scientists working in agriculture, basic research, biotechnology, and medicine shared their group's progress.

CRISPR systems are derived from sophisticated adaptive immune systems found in bacteria and archaea.[4] Besides the commonly used type-II Cas9 protein, other DNA and RNA endonucleases such as Cas12a and Cas13a have been exploited as DNA and RNA editors, and Cas variants have been engineered to recognize an expanded suite of protospacer adjacent motif sequences and function more effectively with improved specificity.[1] However, many novel systems remain to be explored. In this context, dissecting the structure and mechanism of action of various CRISPR systems is important for identifying and applying novel editing tools. For example, Liu *et al.* have solved the structure of a type VI CRISPR-Cas system, LshCas13a, and identified two independent catalytic sites of RNA endonuclease activity, revealing its ability to cleave RNA.[5] Two groups have independently described the DNA cleavage mechanism of the two type V CRISPR-Cas systems, AacCas12b[6] and BthCas12b,[7] which display especially high specificity. These studies provide important insights into the underlying molecular mechanisms of CRISPR systems and establish a framework for modifying them as genome and transcriptome editing tools.

The recent emergence of BEs[8] and PEs[9] with high editing efficiency enables more broad applications in agriculture and therapeutics.[3] In addition to the originally reported rat APOBEC1 (rA1)-Cas9-UGI configuration, Li *et al.* developed Cas12a-derived BEs,[10] while two groups separately developed human APOBEC3A (hA3A)-derived BEs,[11,12] which further expand the BE targeting repertoire to A/T-rich and methylated DNA regions. Furthermore, dual BEs

[1]State Key Laboratory of Plant Cell and Chromosome Engineering, Center for Genome Editing, Institute of Genetics and Developmental Biology, Innovation Academy for Seed Design, Chinese Academy of Sciences, Beijing, PR China; [2]College of Advanced Agricultural Sciences, University of Chinese Academy of Sciences, Beijing, PR China; [3]Gene Editing Center, School of Life Science and Technology, ShanghaiTech University, Shanghai, PR China; and [4]Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, PR China.

that combine both cytidine and adenosine deaminases have been developed and applied in plants[13] and human cells[14] to induce saturated C-to-T and A-to-G mutations at target sites.

Recently, improved prime editing methods have leveraged paired prime editing guide RNAs (pegRNAs) that encode for the same edit.[15] Off-target effects of genome editors have always been of concern. In addition to the off-target effects caused by the permissive binding of gRNA, gRNA-independent off-target effects of BE have been found in plants[16] and mammalian cells.[17] As the cytidine deaminase portion of BE can bind and mutate single-stranded regions in genomic DNA, more advanced versions of BEs have been developed to reduce or eliminate gRNA-independent off-target mutations.[18–20] Of note, PE manifested no observable pegRNA-independent off-target effects in a genome-wide analysis,[21] suggesting high editing specificity. Collectively, the continued improvement of BEs and PEs promises many exciting applications in the future.

Plant Progress

For plant geneticists and crop scientists, time and resources are key elements, limiting crop improvement by conventional breeding. CRISPR and other genome editing technologies provide rapid tools for functional research and molecular breeding. So far, genome editing has been most widely used for gene knockouts to assess function. In 2013, Chinese scientists were the first to produce CRISPR-Cas9-edited rice plants. This approach was extended from dicots to monocots and from diploid to polyploid plants.[22]

Chinese scientists are contributing to the development and application of improved toolkits for single and multiplex knockouts, fragment deletion, gene targeting, base editing, and prime editing using the CRISPR system in plants.[23] Because whole genome-wide mutant libraries are of great value for functional genomics and genetic improvement, two Chinese groups generated large-scale collections of mutants at the whole genome level in rice.[24,25] Insertions or deletions (indels) usually disrupt the expression of genes of interest, but a recent report showed that CRISPR-induced indels in an upstream open reading frame can actually be used to enhance gene translation in plants, thus opening up the application of CRISPR-Cas9 to translational control.[26]

Besides functional genomics research, another advantage of CRISPR in agriculture is to permit accurate and rapid crop improvement by creating disease resistance, herbicide tolerance, and improvements in nutritional value by modifying endogenous genes.[23] Promising results from the use of CRISPR tools in crops and vegetables have already been achieved. One success has been the simultaneous knockout of the three homoeoalleles of the *TaMLO* gene, which conferred resistance to powdery mildew fungus in wheat. This feat illustrated the power of genome editing tools in modifying complex plant genomes and creating crops with valuable traits.[27] Other uses generated simultaneous modification of several meiotic-related genes in rice to form haploid seeds.[28] Although CRISPR approaches have great advantages over conventional breeding, public resistance to and government regulations of the presence of transgenes have impeded its full acceptance. As a result, researchers have employed preassembled Cas9 mRNA/protein-gRNA complexes to express editing genes transiently in bread wheat, providing a potential strategy to address regulatory hurdles.[29,30] Chinese scientists have pioneered many of these CRISPR agricultural advances, which continue to stimulate developments worldwide.

Animal Models and Clinical Trials

Harnessing CRISPR technologies to generate animal models is important for human disease research and therapeutic development. Yan *et al*. were the first to create a huntingtin knockin pig model that mimicked phenotypic features in Huntington's disease,[31] highlighting the importance of using large mammals to investigate neurodegenerative diseases. Monkey models created by gene editing are able to mimic sophisticated features of human cognition and social interactions, providing powerful models for studying such diseases.[32,33]

Another exciting application is the development by Niu *et al.* in producing porcine endogenous retrovirus–inactivated live pigs to serve as a foundational pig strain to provide safe and effective organ and tissue resources for xenotransplantation.[34] Clinical trials using CRISPR-Cas9 systems are underway globally, with China approving the first human CRISPR trial in the world for the treatment of lung cancer in 2016. Subsequently, the United States and EU approved similar clinical trials. In 2019, the effect of knocking out *CCR5* in human hematopoietic stem and progenitor cells (HSPCs) on human immunodeficiency virus infection was tested in another clinical trial, and successful transplantation and long-term engraftment of CRISPR-edited HSPCs were achieved.[35]

Chinese researchers have supported *The CRISPR Journal* since its inaugural issue in 2018, and we are pleased to see more and more Chinese groups submitting and publishing their manuscripts. This special ''CRISPR in China'' issue of *The CRISPR Journal* brings together a diverse selection of original research papers highlighting many interesting new areas of research, from programming cells with CRISPR-associated transposases (see page 350) and CRISPR

editing in *Bombyx mori* (page 371) to rapid adenoviral vector construction (page 381) and a CRISPR strip for COVID-19 point-of-care testing (page 392). We also feature several reports evaluating the CRISPR landscape in China by studying bibliometric and patent data (see pages 313, 321, and 339).

Of course, neither this special issue nor this brief editorial can do justice to the breadth of CRISPR advancements in China. CRISPR technologies are providing inspiration to many areas of research and researchers around the world, stimulating molecular biology research in China and beyond.

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