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Short Communication

CRISPR-Cas Toolkit Expansion in Model Organisms for Cell Development Studies

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DESCRIPTION

The CRISPR (Clustered Regularly Interspersed Short Palindromic Repeat) Cas9 (CRISPR-Associated Nucleus 9) system is ready to change developmental biology by providing a simple, effective method for precisely modifying the gene of any developing organism. This RNA-Guided Nucleus (RGN)-based approach has already been used effectively to induce simultaneously targeted mutations in multiple genes, to create conditional alleles and to generate internally tagged proteins. Explaining the compatibility of RGNs,>20 genes of different plant and animal species as well as multicellular lines and stem cells have been successfully modified. The Current and potential uses of RGNs to investigate genetic function during development.

Research progress has been made so far in CRISPR Cas9, with *Streptococcus piogenes Cas9* (SpCas9) being widely used to achieve effective genetic modification in a variety of species and cell types, including human cell filaments, bacteria, zebrafish, yeast, and mice, fruit fly, roundworm, mice, common crops, pig and monkey. SpCas9 is also dramatically expanding the list of genetically tractable model organisms, for instance, by introducing multiplex mutations into cyanomolgus monkeys.

One of the best challenges with genetically modified animal models generated by zygotic injection of CRISPR factors is genetic mosaicism, partly due to the slow rate of nuclease-induced mutagenesis. Studies to date have generally relied on the injection of Cas9 mRNA into zygotes (single-cell stage fertilized embryos). However, due to the suppression of transcription and translation activity in the mouse zygote, translation into the Cas9 mRNA active enzymatic form may be delayed until after the first cell division.

The modular nature of the two-component CRISPR-Cas9 system and the small size of the target gRNA have the added advantage of being uniquely suited for multiplexing. The use of the common Cas9 nucleus in combination with multiple gRNAs to introduce mutations in several genes simultaneously has been carried out in cultured mammalian cells as well as in genetic sample organisms such as mice, zebra fish and Arabidopsis [1,2].

RGNs have a great ability to differentiate how a gene works during development. As the CRISPR–Cas9 system has been described in more detail recently, will provide a brief overview of the system and focus on some practical observations on the use of RGNs to modify the genome of the developing organism [3]. Genome engineering with RGNs allows direct modification of almost any sequence in a gene to determine its role in development. The need for a specific Protosphere Adjacent Element (PAE) is the main limitation on which genomics can be targeted. PAM is the smallest DNA nucleus adjacent to the Cas9 identification sequence in target DNA and is required for cleavage. The most commonly used *S. pyogenes* Cas9 require PAM sequence 5′-NGG in cell lines, while other PAMs are detected including 5′-NAG, but at lower frequencies [4].

Human pluripotent stem cells are difficult to engineer using classic gene targeting strategies. New avenues of genetic manipulation of these cell types have been opened up by RGNs, which can be easily programmed with different gRNAs. One of the first reports of the use of RGNs for genome engineering demonstrated the success of Induced Pluripotent Stem Cells (iPSCs) with a frequency between 2% and 4% when examined in depth in bulk culture [5].

CONCLUSION

RGNs have made it possible to accurately modify the genes of a wide variety of organisms and cultured cells with unprecedented ease. Improvements for use in diverse organisms, new applications and the rapid speed of adoption make the CRISPR-Cas9 system an exciting and significant technological breakthrough for developing biological studies. Additional methodological progress will undoubtedly further improve the utilization of RGNs. Currently; most RGN-editing experiments take advantage of the NHEJ to create small indels and large clearances that can be used to disrupt genetic expression. Additional improvements in the delivery and/or expression of

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CRISPR-Cas9 components across different systems as well as reduction of off-target effects further enhance the effective use of RGNs. The CRISPR-Cas9 system has the potential to revolutionize developmental biology by sensitively investigating the interaction between genetic activity and developmental events such as cell proliferation, differentiation and morphogenesis.

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