CRISPR/Cas9 for Genome Engineering: the Next Genomic Revolution

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ABSTRACT

Genetically modified cells and animals represent a crucial tool for understanding gene function in development and diseases. The recently developed simple-to-design, easy-to-use and multiplexing CRISPR/Cas9 system is an efficient gene-targeting technology that has already stimulated innovative applications in biology and enabled researchers to make changes in the sequence or expression of any gene in virtually any cell type or organism of interest. When combined with large libraries of sgRNAs, CRISPR/Cas9 enables facile comprehensive forward genetic screens both in vitro and in vivo. Although challenges still remain regarding the off-target mutations, delivery methods as well as the frequency of homology-directed repair, the rapid advance in CRISPR/Cas9 technology opens the door for gene function revealing and genome and epigenome editing. It can be optimistically anticipated that, in the future, CRISPR/Cas9 technology may revolutionize gene therapy research and become a convenient and versatile tool to treat a wide variety of human diseases.

Key words: CRISPR/Cas9; Genome Engineering; Mechanism; Application

DNA-binding domain with a nonspecific cleavage domain from the type II restriction endonuclease FokI, ZFNs can be engineered to target desired DNA sequence and induce DNA double strand break. The nuclease domain requires dimerization to be active, and thus two individual ZFNs must bind opposite strands of DNA with their C-termini a certain distance apart to facilitate dimerization of the nuclease domain. Although ZFNs are effective genome editing tools and numerous innovations to improve the utility of ZFNs have occurred, the technical challenges inherent in designing and validating engineering zinc-finger proteins for a specific DNA locus of interest have limited their use beyond by experts in the field. In 2009, the genome editing field has further expanded with the development of transcription activator-like effector nucleases (TALENs). TALEs are transcriptional activators that specifically bind and regulate plant genes during pathogenesis. Within the TALE structure, a repeated highly conserved 33-34 amino acid sequence with the exception of the 12th and 13th amino acids that mediate DNA recognition. These two locations are highly variable (Repeat Variable Di-residue, RVD) and show a strong correlation with specific nucleotide recognition. Each unit of a TALE protein recognizes only a single base pair. This simple relationship between amino acid sequence and DNA recognition has allowed for the engineering of specific DNA binding domains by selecting a combination of repeat segments containing the appropriate RVDs, which is much easier than zinc fingers to produce and validate, enabling much more widespread applications in genome editing.

The recent emergence of the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 system, Cas9 being the primary enzyme of the CRISPR/Cas system, has opened new doors for genome editing. The CRISPR/Cas9 system is a bacterial defense mechanism that enables bacteria to recognize and cut foreign DNA. CRISPR/Cas9 systems require multiple distinct effectors acting as a complex. CRISPR/Cas9 has been demonstrated to be a simple and efficient tool for genome engineering.

In endogenous CRISPR/Cas9 system, mature crRNA is combined with trans-activating crRNA (tracrRNA), a small RNA that is trans-encoded upstream of the type II CRISPR-Cas locus and is essential for CRISPR maturation by ribonuclease III and Cas9, to form a tracrRNA:crRNA complex that guides Cas9 to a target site. Cas9 unwinds the DNA duplex and searches for sequences matching the crRNA to cleave. Target recognition occurs upon detection of complementarity between a “protospacer” sequence in the target DNA and the remaining spacer sequence in the crRNA. Importantly, Cas9 cuts the DNA only if a correct protospacer-adjacent motif (PAM) is also present at the 3’ end. PAM is a short sequence motif adjacent to the crRNA-targeted sequence on the invading DNA, playing an essential role in the stages of adaptation and interference in type I and type II systems. Different Type II systems have differing PAM requirements. The S. pyogenes system requires an NNGG sequence, while S. thermophilus Type II systems require NNNAGAA. Cas9 is essential for creating DSBs. After binding to the target site, the DNA single-strand matching crRNA and opposite strand are cleaved, respectively, by the HNH nuclease domain and RuvC-like nuclease domain of Cas9, generating a double-strand break (DSB) at the target site. Cas9 induced DSB will trigger cellular DNA repair processes, including homologous end-joining (NHEJ)-mediated error-prone DNA repair and homology-directed repair (HDR)-mediated error-free DNA repair, both of which can be used to achieve a desired editing outcome. In NHEJ-mediated DNA repair, the break ends are directly ligated without the need for a homologous template, generating small insertion and deletion mutations at target sites. These mutations can help us to disrupt or abolish the function of target genes or genomic elements. If the insertion or deletion occurs within a coding exon can lead to frameshift mutations and premature stop codons, gene knockout are achieved. In contrast to NHEJ-mediated DNA repair, HDR-mediated error-free DNA repair requires a homology-containing donor DNA sequence as repair template. The repair template can either be double stranded DNA with homology arms flanking the insertion sequence, or single-stranded DNA oligonucleotides (ssODNs). ssODNs provides an effective and simple method for making small edits in the genome, such as the introduction of single nucleotide mutations. However, HDR is generally active only in dividing cells and typically occurs at lower frequencies than NHEJ.
A dominant mutation in the...

Figure 1 Illustration of CRISPR/Cas9-mediated genome editing. The dual tracrRNA:crRNA is engineered as a single guide RNA (sgRNA), which is a chimeric RNA containing all essential crRNA and tracrRNA components. sgRNA guides Cas9 to a target site when detects complementarity between a "protopspacer" sequence in the target DNA and the remaining spacer sequence in the crRNA. After binding to the target site, the DNA single-strand matching crRNA and opposite strand are cleaved by the HNH nuclease domain and RuvC-like nuclease domain of Cas9 respectively, generating a double-strand break (DSB).

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CONCLUSION

In conclusion, the RNA-guided, two component CRISPR/Cas9 system offers several advantages over the previous protein guided counterparts. The simple-to-design, easy-to-use and multiplexing technology has already stimulated innovative applications in biology and enabled researchers to make changes in the sequence or expression of any gene in virtually any cell type or organism of interest. When combined with large libraries of sgRNAs, CRISPR/Cas9 enables facile comprehensive forward genetic screens both in vitro and in vivo. Furthermore, this gene editing tool has shown therapeutic potentials for genetic diseases, infectious diseases and cancer. Despite the great potential of CRISPR/Cas9 in genome editing, challenges still remain regarding the off-target mutations, delivery methods as well as the frequency of homology-directed repair. However, with the rapid advance in CRISPR/Cas9 technology, it opens the door for gene function revealing and genome and
epigenome editing. It can be optimistically anticipated that, in the future, CRISPR/Cas9 technology may revolutionize gene therapy research and become a convenient and versatile tool to treat a wide variety of human diseases.

CONFLICT OF INTERESTS
The authors have no conflicts of interest to declare.

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