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Principles and Significance of Base Editing and Prime Editing

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Abstract

In this concise commentary we introduce readers to the concepts and principles of "Base editing" and "Prime editing" – two very important methodologies that nearly revolutionized the field of Genome Editing over the past few years.

Keywords: Crispr • Genome editing • Base editing • Prime editing

Introduction

Classical Crispr-Cas9 Editing

CRISPR-Cas9 relies on non-homologous end joining (NHEJ) or homology-directed repair (HDR) following generation of double-strand breaks by nucleases, such as Cas9. HDR allows precise editing by donor DNA. However, precision of this method is not perfect – sometimes, it leaves uncorrected sequences at double-strand breaks and can result in integration in off-target sites [1-18].

Base Editing

This method was originally developed by Alexis Komor, David Liu and colleagues at Liu lab. To date, two major classes of Base Editors have been described: cytosine base editors (CBEs), which change C•G base pairs to T•A (Komor, Kim, Packer, Zuris, & Liu, 2016) and adenosine base editors (ABEs), which convert A•T base pairs to G•C (Gaudelli et al. 2017).

Base editor is designed so that there is a fusion between deaminase and Cas9 nickase or catalytically inactive dead Cas9. Deaminase functions to deaminate cytosine (C) and adenine (A) bases. Other base conversions are not currently possible with this method, as well as targeted deletions, insertions or some other types of mutations.

This system is tethered to a single-stranded DNA (ssDNA)-modifying enzyme. Cas protein makes a complex with a user-programmed guide RNA (gRNA) and binds to a genomic locus (termed the "protospacer") that is complementary to the sequence of the gRNA and harbors a protospacer-adjacent motif (PAM, a short DNA sequence specific to the Cas protein used; Upon binding, the Cas protein locally denatures the target double-stranded DNA region to form an R-loop, exposing a small window ~5 nucleotides (nt) long of ssDNA (Jiang et al., 2016). Once bound, the ssDNA-modifying enzyme catalyzes the deamination of target nucleobases within this window. Subsequent DNA replication or repair of these modified base intermediates (uracil or inosine) results in permanent introduction of single-base substitutions. Base Editor system is being currently optimized by Alexis Komor lab at UC San Diego.

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There are many advantages in this method. Sometimes it out-performs Prime Editing. Still there are certain limitations of this method that preclude applying base editing approach as a strategy to cure many genetic diseases. It does not allow certain genetic engineering applications, including molecular tagging.

Prime Editing

This method has been developed more recently in the laboratory of Professor David Liu and being optimized by David Liu's and other laboratories. This method does not require double-stranded DNA breaks.

Prime Editors consist of Cas9 H840A nickase fused to M-MLV reverse transcriptase (RT) and prime editing guide RNA (pegRNA). pegRNA includes primer binding sequence (PBS) and RT template sequence with the desired edit.

Peg RNA 1) guides Cas9 H840A nickase to genomic DNA sequence to be edited; 2) determines the edited sequence to be inserted. Cas9 nicks one strand, RT fused to Cas9 copies edited sequence at the nicked target site, then cell uses DNA repair machinery to incorporate the edited sequence, making permanent change in genomic DNA.

The steps of editing by Prime Editor:

1) Upon target recognition, the protospacer adjacent motif (PAM)–containing strand is nicked by Cas9 H840A nickase that makes single-stranded break in one of the strands (non-target strand);
2) Primer binding sequence (PBS) of pegRNA hybridizes with 3'-region of the nicked strand;
3) Reverse transcription of the template target sequence (3'-flap edit);
4) Ligation and incorporation of newly synthesized sequence;
5) Edited sequence is incorporated into both strands by DNA repair.

Conclusion

The advantage of Prime Editor method is that it has broad range of applications including various types of mutations, insertions, deletions, combinations of several mutations. For example, insertions and deletions of up to 80 nucleotides could be useful for genetic engineering. It is also an advantage that this method does not involve generation of double-stranded breaks.

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