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Prime Editing Reshapes Plant Genetics with Precision Genome Modification

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Abstract

Prime editing (PE) represents a cutting-edge genome editing technique allowing for precise alterations in mammalian genomes, including all potential types of point mutations and minor insertions and deletions. Utilizing a Cas9 nickase fused with a specially engineered reverse transcriptase (RT), PE operates via a prime editing guide RNA (pegRNA) to direct the desired edits. Versions such as PE2 and PE3 enhance editing efficiency, while PE3b mitigates indel formation. In agricultural contexts, PE has been successfully employed in rice, wheat, tomato, maize, and legume crops, showcasing its versatility. Despite its advantages in minimizing off-target events, PE faces challenges such as lower efficiency and strict PAM requirements, necessitating further research to optimize its potential for broader applications, including in vivo editing and complex mutations.

Introduction

Many genome editing applications, especially those involving the correction of harmful mutations in mammalian genomes, often require the introduction of specific point mutations, small insertions, or deletions. CRISPR-Cas nucleases create double-strand breaks (DSBs) and can be employed to disrupt, insert, or remove DNA sequences using repair processes, or to make precise alterations using homology-directed repair (HDR) in suitable cell types with high levels of accompanying byproducts. Base editors can introduce transition point mutations without causing DSBs but currently cannot create transversion point mutations or precise insertions or deletions. Additionally, base editors may produce unintended mutations when multiple target nucleotides are present within the editing window, and availability of a suitable PAM sequence may sometimes limit targeting certain bases. Prime editing represents a modern genome editing technique that allows for the introduction of all 12 potential types of point mutations, including all 6 possible conversions of base pairs, as well as minor insertions and deletions, in a precise and targeted manner with favorable editing to indel ratios. This method serves as a versatile and accurate tool for genome editing, enabling the direct insertion of new genetic information into a designated DNA target site.

How a prime editing works?

Prime editor has a Cas9 nickase (nCas9; H840A) fused with a specially engineered reverse transcriptase (RT). The RT is guided by a prime editing guide RNA (pegRNA) that specifies the target site and encodes the desired edit. The pegRNA is essentially a modified single guide RNA (sgRNA) with a 3' extension containing the RT template and primer-binding site (PBS) sequences. The nCas9, which is a Cas9 variant with reduced catalytic activity due to the H840A mutation, is employed to nick the editing strand of the double-stranded DNA target. Subsequently, the nicked strand serves as a primer for reverse transcription of an edit-encoding extension (RT template) on the pegRNA directly into the target site. This process generates a branched intermediate consisting of two competing single-stranded DNA flaps. The 3' flap harbors the edited sequence, while the 5' flap retains the unedited sequence. In mammalian cells, the 5' flap is preferentially cleaved by structure-specific endonucleases like FEN1 or 5' exonucleases such as Exo1. The ligation of the 3' flap incorporates the edited DNA strand into the heteroduplex DNA, which contains one edited strand and one unedited strand. Finally, the DNA repair machinery permanently installs the desired edit by copying the information from the edited strand to the complementary strand to resolve the heteroduplex.

Versions of prime editing systems

Prime editor 1 (PE1)

PE1 contains a Cas9H840A nickase fused with a wild-type Moloney murine leukemia virus reverse transcriptase (M-MLV-RT) at the C-terminal.

Prime editor 2 (PE2)

Prime editor 2 (PE2) utilizes an engineered M-MLV-RT Penta mutant (D200N/L603W/T330P/

T306K/W313F) which enhances thermostability, processivity, DNA-RNA substrate affinity, and deactivates RNase H activity. PE2 demonstrates approximately threefold higher editing efficiency in human cell lines compared to PE1.

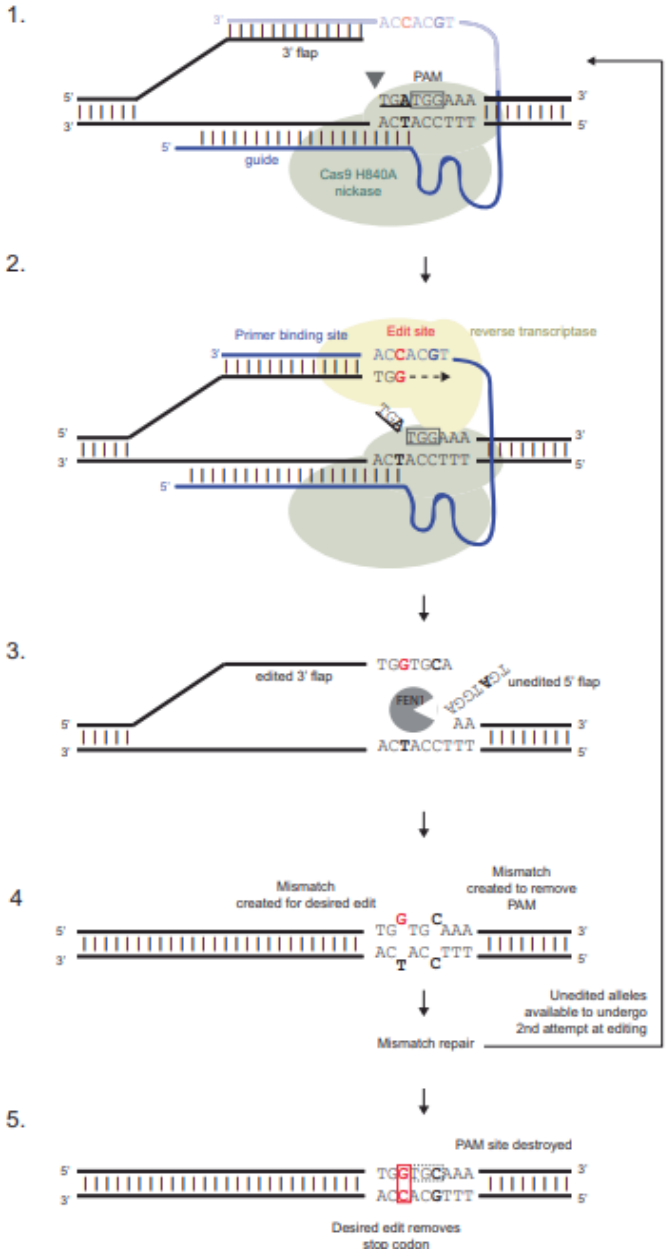

Figure1: Mechanism of prime editing (Source: Scholefield *et al.*, 2021).

Prime editor 3 (PE3)

PE3 enhances editing efficiency in human cells by two to fourfold compared to PE2 by nicking the non-edited strand to stimulate DNA repair mechanisms. PE3 employs the double nicking of two complementary DNA strands, potentially leading to double-strand breaks (DSBs) and subsequent indel formation through non-homologous end joining (NHEJ) repair. To mitigate this, a variation of the PE3 system, known as prime editor 3b (PE3b), has been developed. PE3b employs a nicking guide RNA (gRNA) with a protospacer that matches the edited strand but not the original allele, resulting in a thirteenfold reduction in the average number of indels compared to PE3 in human cell lines, while maintaining editing efficiency.

Prime editing for crop improvement

The rice ALS gene was modified by introducing two nucleotide changes: one from G to T, converting tryptophan 548 to leucine, thus conferring resistance to the herbicide Bispyribac sodium for controlling broad-leaf weeds after emergence; and another silent change from G to A, which disrupts the PAM site to prevent repeated targeting of the same site e (Butt et al., 2020). There are applications in plants besides those in rice and wheat. The codon and promoter alterations significantly increased the efficiency of prime editing in tomato, to levels comparable to those in rice (Lu et al., 2021). A prime editing vector with two pegRNA variants for the W542Land S621I double mutations in ZmALS1 and ZmALS2 was constructed by Y. Y. Jiang et al. (2020), which demon-strated the applications of prime editing in maize. Similarly, Biswas et al. (2022) successfully edited a mutant GFP in rice, peanut, chickpea, and cowpea protoplasts.

Merits and drawbacks of PE

Prime editing (PE) offers distinctive benefits, broadening the range of edits to encompass all base-to-base conversions, as well as small insertions and deletions. Moreover, prime editors have the ability to introduce point mutations at distances exceeding 30 base pairs from the Cas9 nicking site, providing greater flexibility in targeting compared to Cas9-mediated HDR with additional donor templates. PE also demonstrates effectiveness in minimizing off-target events, potentially attributed to three nucleic acid hybridization steps. These steps involve conventional annealing between the protospacer and spacer sequence, nicking of the non-target strand by nCas9, and hybridization of the PBS and 3' end of pegRNA to the target strand, thus reducing the likelihood of off-target loci.

Each tool has its own advantages and limitations. Prime editing (PE) is still in its early stages, with several important considerations. Compared to current base editing methods, PE may be less efficient and produce more byproducts, though it surpasses traditional editing techniques. Additionally, the strict PAM requirement of SpCas9-based proteins in PE poses limitations on target sequences, potentially reducing efficiency and usability. Further exploration is needed to assess PE's versatility for in vivo editing and its ability to address complex mutations.

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