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Prime Editor: A New Precision Genome Editing Tool

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SUMMARY

Double Stranded Breaks (DSBs) are key events in genome editing, but they carry the risk of genome instability and unpredictable outcomes of DNA repair. Therefore, approaches to alter the targeted DNA or gene expression without inducing DSBs have been explored. Cytidine deaminase-incorporating DNA base editors (CBEs) have been developed for nucleotide conversion from C to T, and adenine deaminase-based DNA editor (ABE) has been developed for A to G conversion. These base editors, however, can edit a single base at a time. Recently developed prime editing technology can introduce InDels and all 12 base-to-base conversions (both transitions and transversions) without inducing DSBs. Prime editing technology has potential for application in gene correction and knockouts, protein engineering and directed molecular evolution by virtue of which it can do wonders in the field of plant biology.

INTRODUCTION

Owing to the adverse climatic conditions and different biotic and abiotic stresses there is an urgent need to breed the crops for the foreseen challenges. The conventional crop breeding methods, though being utilized since decades, have certain limitations. The crop breeding approaches like cross breeding and mutation breeding take longer time and demand handling of large populations making it labor intensive. Transgenic breeding, though takes lesser time to develop promising variants, is withheld by regulatory issues from utilization in crop improvement. Genome editing technologies have recently been established as powerful tools for creating targeted mutations in crop plants. The CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein) system enables plant breeders to control the specific introduction of targeted sequence variation by making double stranded breaks (DSBs) in the genome of the plant. However, CRISPR/Cas system lacks the ability to make predictable changes making it unsuitable for curing the gene sequences (Chen et al., 2019). Base editing technology enables conversion of a specific base into another with utmost efficiency. Cytosine base editors (CBEs) and adenine base editors (ABEs) mediates conversion of CG into TA base pair, and AT into GC base pair, respectively (Sutar *et al*., 2020). By editing single base C to T in Acetolactate synthase (ALS) gene, a herbicide tolerant watermelon variety was developed (Tian *et al*., 2018). Base editing can bring about only transition mutations and cannot insert or delete a stretch of sequence in the genome.

Prime Editing

Prime editing technology introduces InDels and all 12 base-to-base conversions (both transitions and transversions) without inducing DSBs (Anzalone *et al*., 2019). It is a promising technology that can cure the undesired changes occurred in the wild type alleles, resuming the normal functioning of the gene. Prime editor breaks the bottleneck of earlier genome editing technology, making it a highly promising approach for advanced breeding.

Mechanism of Prime Editing

Prime editing uses Cas9 nickase fused to reverse transcriptase and engineered gRNA (a prime editing guide RNA, pegRNA), which consists of a primer binding site (PBS), the desired edited sequence, and a sequence that recognizes the target DNA. The Cas9 nickase is recruited to the target DNA sequence by pegRNA, then nicks the PAM-containing DNA strand. The 3′ end of the nicked DNA strand hybridizes to the PBS of pegRNA, priming reverse transcription of the desired edited-sequence on the pegRNA by reverse transcriptase fused to Cas9 nickase. Hybridization between the target DNA and the reverse transcription product produces a 3′ flap with edited-sequence or 5′ flap with unedited sequence. The 5′ flap is cleaved preferentially by the endonuclease, and the 3′ flap is ligated to the DNA strand (Figure 1). The heteroduplex DNA is repaired by the endogenous DNA repair process, resulting in stable incorporation of the edited sequence into the genome.

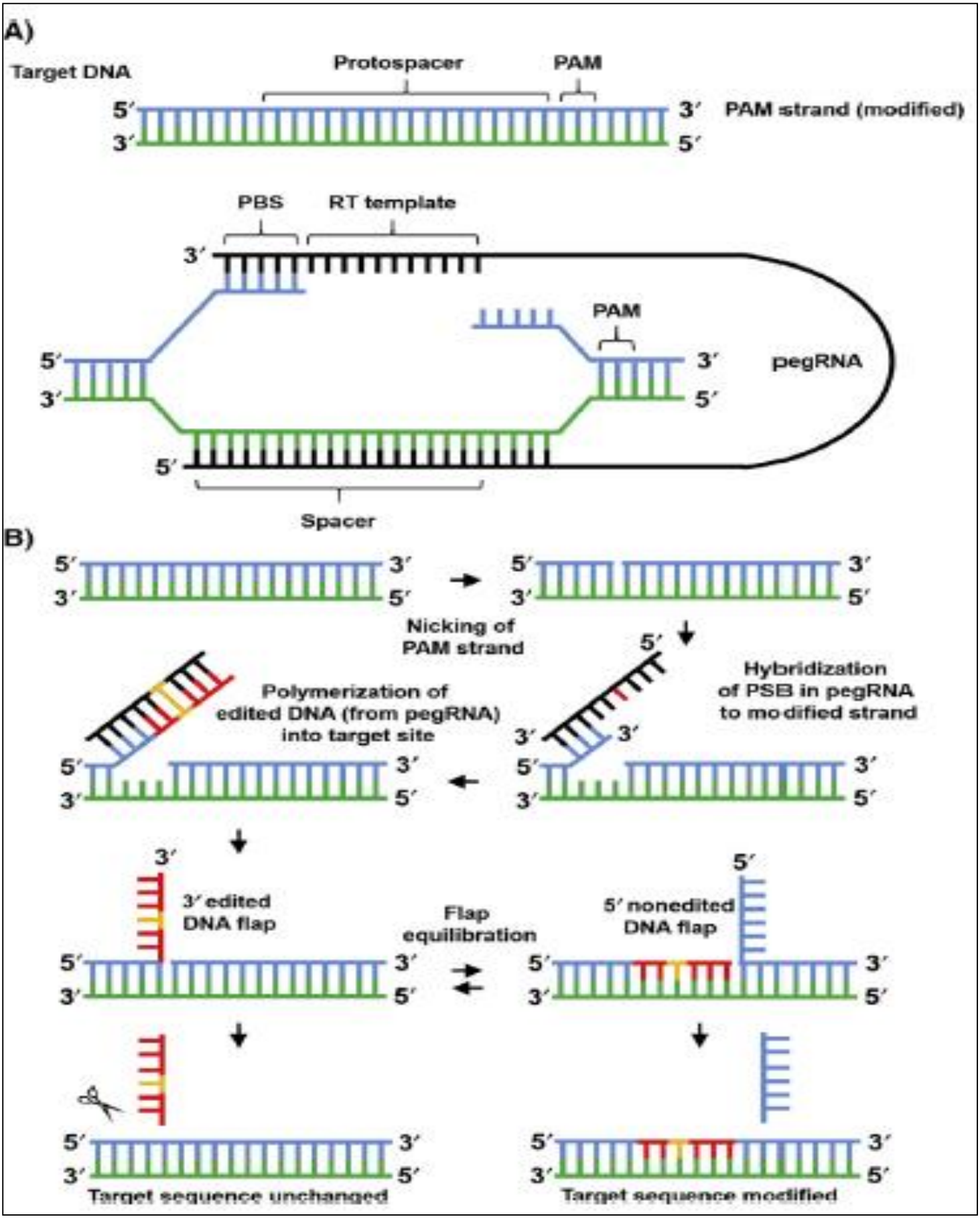

Figure 1. Prime Editing System (Source: Marzec *et al.*, 2020)

CONCLUSION

Prime editing would be a promising technology for plant genome engineering, especially because prime editing can achieve efficient knock-in of DNA fragments in plant cells. Generally, HDR efficiency is low in plant cells, so knock-ins of DNA fragments to target sites is difficult. However, prime editing offers a new strategy for knock-in of DNA fragments via an HDR-independent pathway. Genetic modifications like introduction of single and multiple point mutations, introduction or replacement of desired amino acid, introducing premature stop codon, correction of undesired mutation, and insertion, deletion or substitution of small DNA segment can be brought about using this technology.

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