DITING WITHIN THE FRAMEWORK OF USING PRIME EDITING APPLICATIONS.

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Abstract

First-order editing is a precision CRISPR-based “search-and-replace” technology that initially damages only one strand of DNA. The system introduces the edit into one strand via reverse transcription, creating a temporary mismatch before the cell’s natural DNA repair resolves the heteroduplex. Strategies such as PE3 use a secondary nick on the unedited strand to correct the mismatch.

Keywords: functional genomics; genome-editing technology; multiplexed assay of variant effect; precision medicine; prime editing; saturation mutagenesis.

Introduction

The key features of the mechanism are valid today.

Single-stranded nicking: Unlike classical CRISPR-Cas9, which causes double-strand breaks, the base editor uses an engineered Cas9 nickase to open only one strand at the target locus.

Single-strand nicking prevents double-strand breaks (DSBs). By using an engineered Cas9 nickase (such as a D10A mutant), the base editor opens only one strand at the target locus. This targeted single-strand break (SSB) or "nick" avoids the genotoxicity and unpredictable insertion/deletion (indel) mutations tied to classic DSB repair.

How the Mechanism Works

Targeted Nicking: The engineered Cas9 nickase uses its guide RNA to find the specific locus and cuts only the non-edited DNA strand, leaving the complementary strand intact.

Enzymatic Conversion: A fused deaminase enzyme (such as cytidine or adenine deaminase) directly alters a specific base on the exposed single strand.

Cellular Repair: The "nick" signals the cell's repair machinery to replace the unedited strand, permanently locking in the corrected base pair without ever severing the DNA helix.

Template-based synthesis: A fused reverse transcriptase reads a prime editing guide RNA (pegRNA) to synthesize a new genetic sequence directly on the unwound single-stranded strand.

This process describes Prime Editing (a specialized CRISPR method), where a fused nCas9-Reverse Transcriptase uses a pegRNA (prime editing guide RNA) to write new genetic sequences without making double-strand breaks.

Step-by-Step Mechanism

Targeting & Nicking: The pegRNA guides the protein complex to the target DNA, where the Cas9 nickase creates a single-strand "nick".

Priming: A specific section of the pegRNA (the Primer Binding Site, or PBS) pairs with the unwound, single-stranded DNA.

Synthesis: The fused Reverse Transcriptase (RT) reads the RNA template on the pegRNA and synthesizes a new DNA sequence directly onto the genomic DNA.

Resolution: The newly created DNA flap is integrated into the genome through cellular repair pathways.

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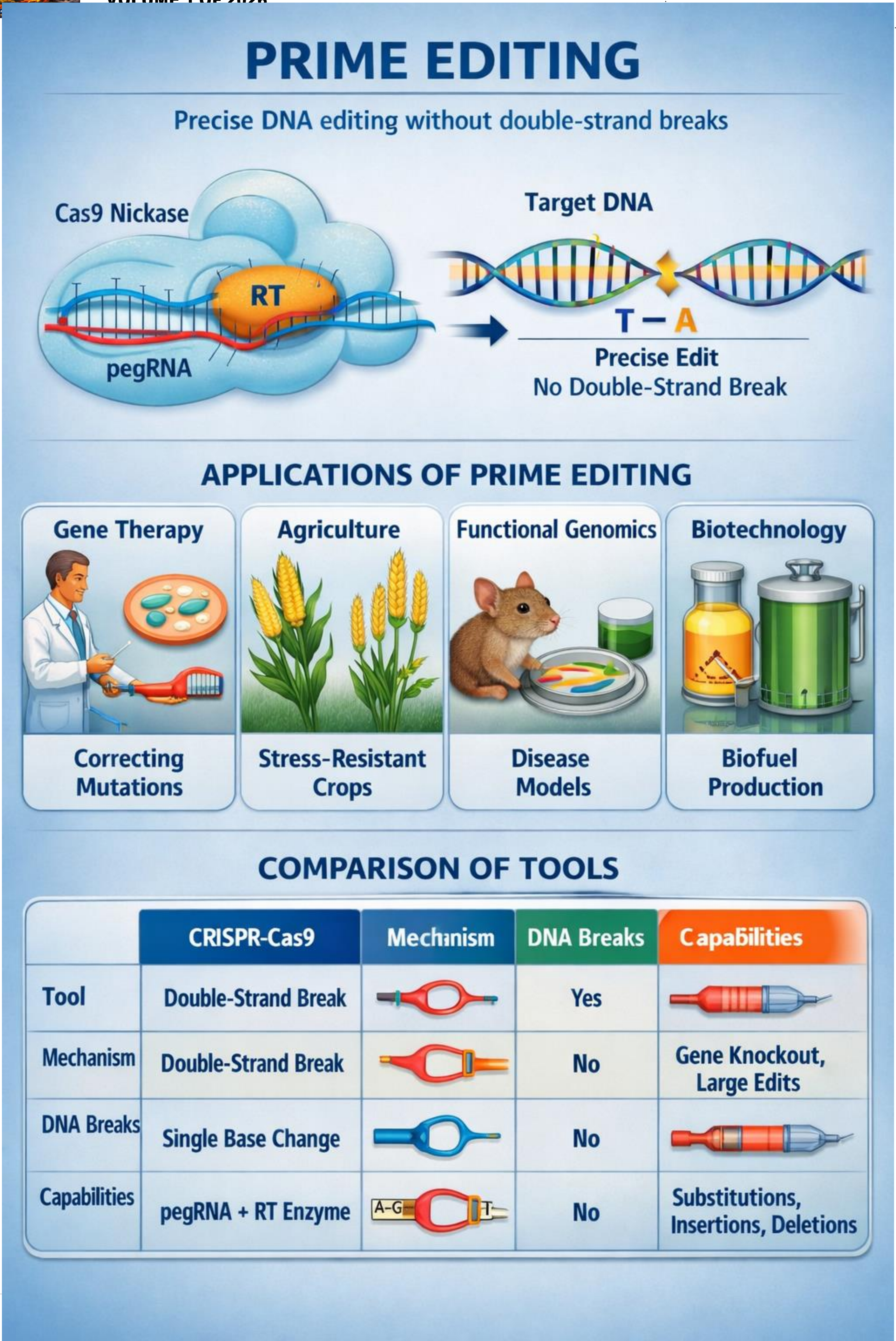

Learn about Template-jumping prime editing for larger genetic insertions.

Review compositions and methods in Google Patents.

Explore structural mechanisms in PubMed Central (PMC).

Mismatch repair solution: The edited single-stranded flap is integrated, leaving a temporary mismatch between the two strands. The cell's mismatch mechanism then copies the edited change to the complementary strand to ensure stability.

This cellular mechanism harnesses the natural, evolutionary-conserved DNA mismatch repair pathway to finalize gene editing. By exploiting the cell's natural bias to copy edited information from a newly integrated single-stranded flap to the complementary strand, it achieves permanent genetic modification.

The standard DNA mismatch repair process relies on several primary components to operate:

Recognition: The MutS homolog complex identifies and binds to the distortion created by the temporary mismatch.

Signaling & Excision: The MutL complex gets recruited to activate endonucleases, directing the cell to copy the newly introduced edits rather than deleting them.

Resolution: Polymerases and ligases then resolve the heteroduplex, ensuring stability.

Further Exploration: Mechanistic Insights

Learn how cells modulate mismatch repair from PubMed Central (PMC).

Explore advanced editing techniques via MDPI.

Sequential Double-Nicking (PE3): To force this permanent fixation, the system can sequentially nick the complementary strand to stimulate repair using the newly edited strand as a template.

In Prime Editing (PE3), the system introduces a second nick on the non-edited complementary strand. By directing cellular repair machinery to degrade the unedited strand, the cell is forced to synthesize a replacement using the newly edited strand as a template, substantially boosting editing efficiency.

How the Mechanism Works

Initial Nicking & Extension: A prime editing guide RNA (pegRNA) directs a Cas9 nickase fused to reverse transcriptase to nick the target strand. The primer binding site (PBS) hybridizes, and the edit is synthesized.

Second Nicking (PE3): An additional nicking sgRNA targets the unedited complementary strand, typically 14 to 116 nucleotides away from the initial site.

Strand Bias: Nicking the complementary strand triggers the mismatch repair (MMR) pathway. The cell recognizes the nicked, unedited strand as damaged and degrades it, subsequently repairing the gap by copying the complementary edited strand.

Key System Variations

PE3 vs. PE2: PE2 lacks the second nicking sgRNA, leaving heteroduplex resolution to chance. PE3's sequential double-nicking increases editing efficiency 2–3 fold.

PE3b: To reduce unintended insertion/deletion (indel) byproducts, PE3b utilizes a nicking guide designed so its spacer sequence only matches the edited allele. It only nicks the complementary strand after the edit has been successfully incorporated.

PE4/PE5: These variants incorporate an engineered dominant-negative mutant protein (MLH1dn) to suppress the MMR pathway temporarily, boosting precision and reducing byproducts when double-nicking.

Outlook:

Sequential triple excision (3PE3): To force this permanent fixation, the system can sequentially incise the complementary strand to stimulate repair, using the newly reacted strand as a template, but if a mutation occurs it will be removed and the next repair 3 will replace the mutation.

This process resembles aspects of **mismatch repair (MMR)** and **base excision**

repair (BER), both of which rely on strand discrimination and template fidelity. In synthetic biology or CRISPR-based editing, similar logic applies: the edited strand becomes the "truth," and repair pathways overwrite the complementary strand until both match.

Future prospects:

Advanced genomic engineering. Overview of universal mechanisms. Understand the role of DNA repair pathways with a review of PMC mechanistic findings.

Advanced genomic engineering relies heavily on DNA repair pathways, which act as universal mechanisms for maintaining genome integrity and enabling precise editing. Recent PMC reviews highlight how cells balance error-prone and error-free repair routes, and how CRISPR-Cas systems exploit these pathways to achieve targeted modifications.

Universal DNA Repair Mechanisms

- **Base excision repair (BER)** Corrects small base lesions caused by oxidation, alkylation, or deamination. Essential for maintaining fidelity in engineered genomes.
- **Nucleotide excision repair (NER)** Removes bulky DNA adducts and UV-induced lesions. Plays a role in protecting engineered cells from environmental mutagens.
- **Mismatch repair (MMR)** Detects and fixes replication errors. In genome engineering, MMR influences mutation fixation and editing efficiency.
- **Double-strand break repair (DSBR)**
- **Homologous recombination (HR):** Error-free, template-dependent repair. Critical for precise gene insertion in CRISPR editing.
- **Non-homologous end joining (NHEJ):** Error-prone, template-independent repair. Often used for knockout mutations.
- **Alternative pathways (MMEJ, SSA):** Error-prone but sometimes

exploited for specific editing outcomes.

Conclusions

Role in Genomic Engineering

- **CRISPR-Cas9 and related nucleases** induce double-strand breaks (DSBs). The cell's choice of repair pathway determines whether the outcome is a **precise insertion (HR)** or a **disruptive mutation (NHEJ/MMEJ)**.
- **Pathway biasing strategies** (e.g., synchronizing cells in S-phase to favor HR, or inhibiting NHEJ factors) are actively researched to improve editing accuracy.
- **Emerging players** such as **long non-coding RNAs, sirtuins, circadian regulators, and heat shock proteins** modulate DNA damage response and may be harnessed to fine-tune repair outcomes in engineered systems.

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