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How CRISPR Prime Edited Gene Drives could lead to a Genetic Revolution

Benjamin Clifford
Southern Maine Community College, benjaminfclifford@smccme.edu

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Introduction

Clustered regularly interspaced palindromic repeats (CRISPR) was discovered in 1987, this gave us an ability to affordably modify genetic material within a genome. Previously, CRISPR associated protein 9 (Cas9) cut both DNA strands at a specific location determined by a guide RNA. The strands will repair the break with two main mechanisms homology-directed repair (HDR) and non-homologous repair (Fig 1.). Unfortunately, introducing a designed sequence is not easy. This review describes two new applications of CRISPR as a technology. A new technique was introduced last year called CRISPR Prime Editing. Another recent application of CRISPR is gene drives. With help from CRISPR prime, an allele can be designed to become homozygous by homologous recombination as gametes form so that the designed allele will nearly always be passed to progeny. It may be possible to accurately identify and correct genetic defects in a population. These new technologies create incredible possibilities, as well they also raise major ethical questions.

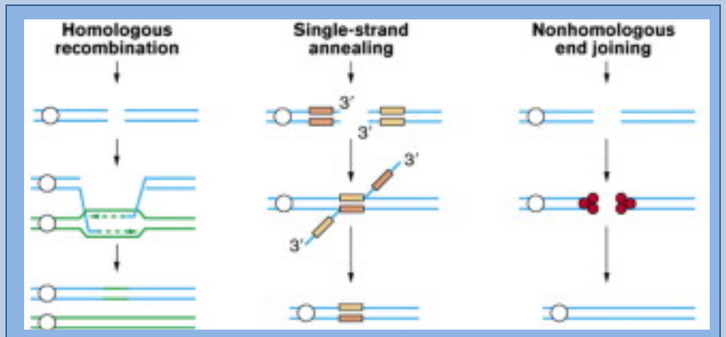

Figure 1: Homologous recombination (left) which is the use of a similar strand as a template to copy for the repair, and the single-strand annealing pathway (center) which is when there are two similar sequences on opposite sides of the break, the sequence causes a d-loop to form to copy the leading strand, this strand anneals and the lagging strand copies the complementary sequence which completes the repair. Then non-homologous end joining: (right) which is the direct ligation of the two strands without any template or manipulation(right) .

Objective

The objective of this literature review is to understand multiple current concepts and how they could be used in combination to enhance genetic modification. In this review I focused on CRISPR prime editing and gene drives.

References

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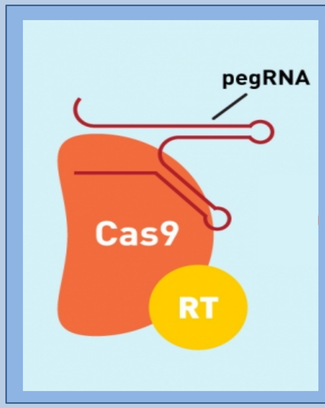

Figure 2: A prime editing molecule is comprised of a prime editing guide RNA (pegRNA), a modified Cas9 enzyme, and a reverse transcriptase (RT) to create a DNA sequence from the engineered guide RNA.

CRISPR Prime Editing

Prime editing is a new advancement from the original CRISPR process. With prime editing, there is more accuracy and more success than previous methods. Prime editing utilizes an engineered pegRNA to guide the modified Cas9 enzyme, reverse transcriptase, and the desired insert sequence to a specific location. This has a much higher success rate than previous methods which can give us the ability to make extremely accurate edits at a relatively affordable and efficient rate.

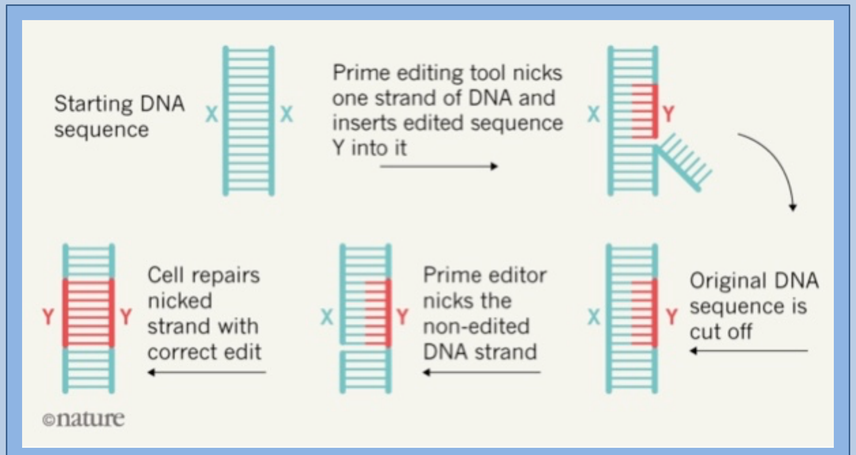

Figure 3: The step by step process of CRISPR prime editing. The Cas9 nicks one strand at a specific location, reverse transcriptase then inserts the new sequence from the provided RNA sequence. Once the new sequenced has ligated the Cas9 cuts off the original sequence, and then nicks the other backbone at the same location. The process then finalizes the other strand.

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Gene Drives

A gene drive propagates an allele of a gene throughout a population in a much shorter time than it would spread with Mendelian genetics. When dealing with modified genes if the mutated parent breeds with a wild type parent the offspring will have a fifty percent chance of inheriting those mutated genes. In three generations as long as only wild type partners are selected the modified gene will be bread out of the population. Gene drives force inheritance and it gives the offspring a higher percent chance of being inherited. Unfortunately with the ability of these genes to be modified through inheritance, there is the ability of the offspring to develop a resistance to modification. This can create an issue and needs to be researched further on how to prevent that resistance from happening.

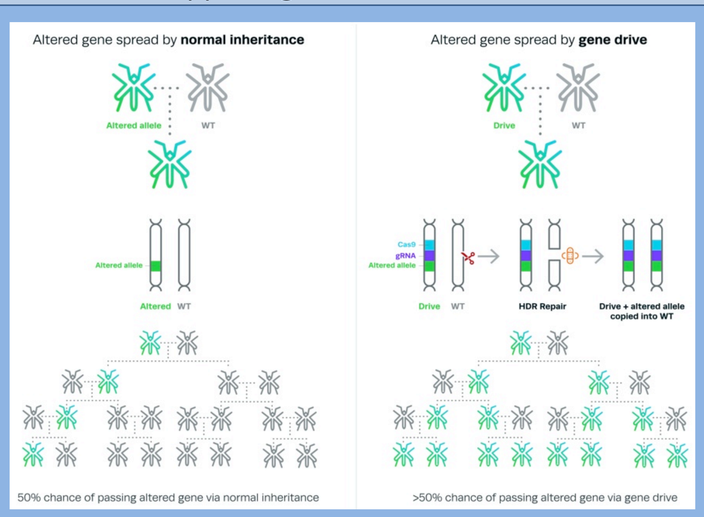

Figure 4: Compared inheritance between normal and mutated gene drive inheritance. The blue flies are wild type nonmutated species and the red flies are the gene drive mutations. By the F3 progeny altered gene drives dominate the population.

Combined Technology

The ability to successfully and accurately modify a genome, while having that correction be an engineered insertion paired with the ability of gene drives to push mutations through a population aggressively and rapidly could lead to a genetic revolution. We would have the ability to wipe out certain genetic diseases from our population. The ability to combine these two technologies has endless possibilities. Before further research, it would be relevant to determine the ethical elements and possible ramifications from the use or abuse of this technology.