Genome Editing and Frontiers in Bio-Engineering

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Abstract:- **The advances in genetic analysis and manipulation innovations in high- throughput DNA sequencing and editing have been felt broadly and globally, starting from the work on model organisms, evolutionary studies, agriculture to medical applications. Genome editing has become a very important tool in research, this technique can specifically modify individual nucleotides in the genome of living cells, monitor and reduce off target effects, and this actually brings new opportunities within range. The genome editing has a general applicability in microbes, plants, human cells and animals; this makes it have a very wide range of potential uses in tackling societal problems. The potential applications of genome editing include, but are not limited to gene and cell based therapies to control diseases and in reproduction, preventions of inheritance of disease traits, control of vector borne diseases, improved crop and livestock breeding techniques, improved animal welfare, modification of animal donors foe xenotransplantation, industrial microbial biotechnology to generate biofuels and efficient production of pharmaceutical drugs and other high value chemicals. The advent of genome editing technology has evoked many enthusiasm and controversy in the contrary. Many concerns have been expressed especially in the African context for example, the technology is not natural and there is gaps in our technical knowhow that the impacts are uncertain and may be inequitable, the current regulations cannot keep pace with the rate of technological innovations. The current knowledge gaps and uncertainties emphasize the need for more research with the expectations that the research advances will fill many of the current knowledge gaps and the progressive refinement of the genome editing tools to ensure efficiency and specificity, thereby reducing off-target effects. And addressing aspect of further mutations. The genome editing technocrats and regulators should ensure research applications is evidence-based, takes into account likely benefits as well as hypothetical risk and is proportionate and sufficiently flexible to cope with future advances. Going forward it's worth noting that the latest genome editing technologies has resolved many issues making direct and precise genetic manipulations possible in essentially types of cells and organisms. It is key to mention that progress in science depends on new techniques, novel discoveries and ideas. This review paper aim to examine the origin of genome editing platforms and speculate about where we are headed through the application of these new technology of genome editing.**

Keywords: **Genome editing, DNA sequencing, nucleotides**

I. INTRODUCTION

Genome editing is also known as the gene editing is a technologies that give scientists the ability to change an organism's DNA. These technologies allow alteration of a targeted DNA sequence by cutting the DNA molecule at a selected point. This enable genetic material to be added, removed, or altered at particular locations in the genome. There are many approaches that has been developed, a recent one is known as CRISPR-Cas9, which is short for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9. The advent of the CRISPR-Cas9 system has generated a lot of excitement in the scientific community because it is faster, cheaper, more accurate, and more efficient than other existing genome editing methods (1).Genome editing is different from previously employed techniques of bioengineering since the DNA template can be introduced more efficiently and precisely at the molecular level. However, researchers need to do more in many cases to better understand the biological consequences of the nucleotide changes.

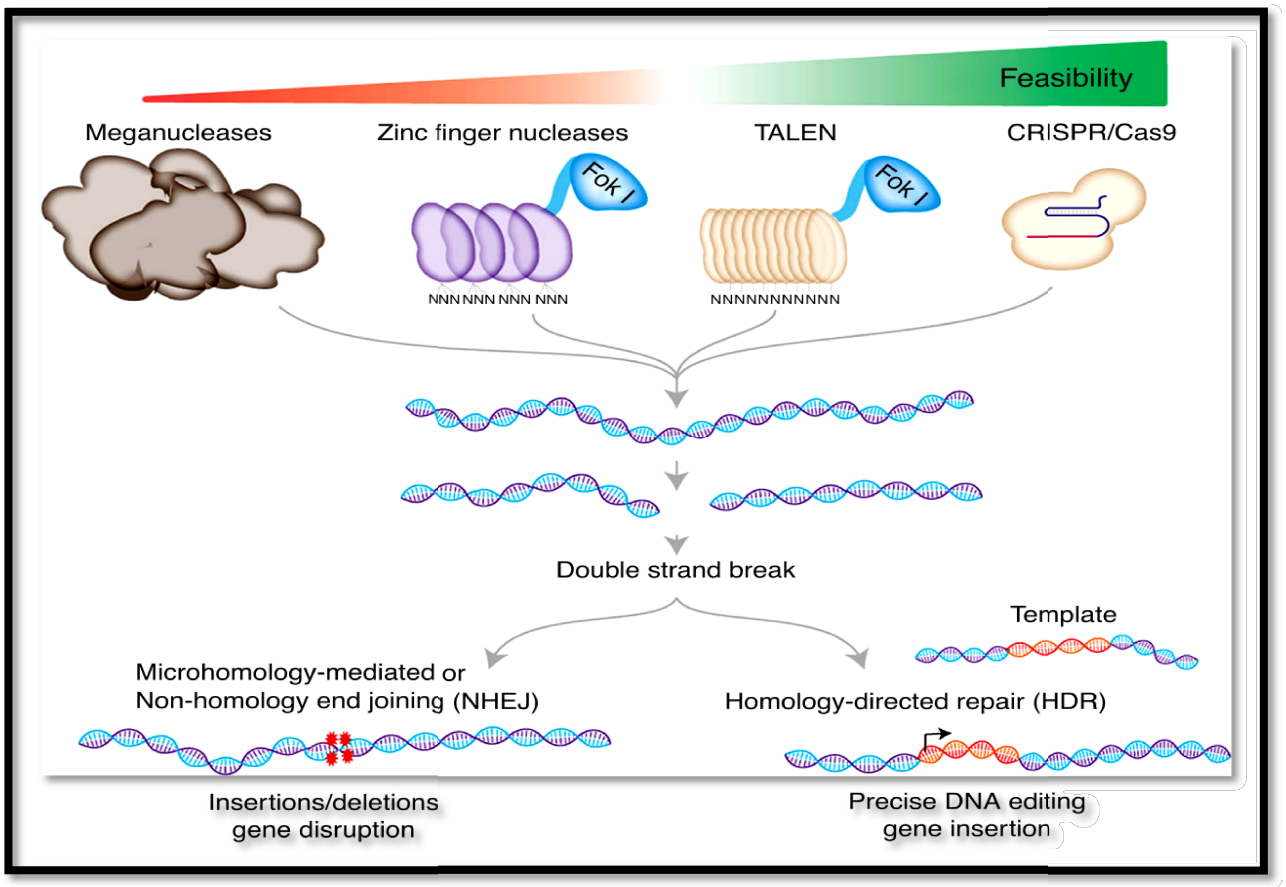
The genome editing technology is advancing rapidly and the technology has reached an already sufficient threshold to warrant a proportionate, robust and flexible management of research and innovation, however there are relevant matters relating to the regulation of new products and the avoidance of harm, whether harm is caused inadvertently to human health and the environment or by intended misuse, dual usage with biosecurity consequences (2). It is important for the regulatory fraternity to establish a significant strengths in genome editing research; which is rigorous, risk-benefit assessment as part of the regulatory process that any safety concerns are addressed appropriately and research outputs can be translated into new product and service to fulfill the continent needs. The potential benefits of genome editing technology are immense; microbial biotechnology, in the provision of more efficient pathways for biofuel synthesis high-value chemicals and pharmaceuticals; new vehicles for drug delivery; sensors and environmental remediation; plant and animal breeding in precision agriculture to tackle issues of food and nutrition security, animal health and a more sustainable agriculture; and a range of other human health applications (3). Tackling disease, genome editing of human cells brings opportunities to treat or avoid monogenic disorders (with recent research in cystic fibrosis, Duchenne muscular dystrophy, diseases affecting the immune system and haemophilia (4) and infectious disease (with first studies in human immunodeficiency virus (HIV)) and diseases that have both a genetic and an environmental component (5).

II. GENOME EDITING PLATFORMS

Genome editing is different from the early genetic engineering techniques that randomly inserts genetic material into a host genome, since it targets the insertions to site specific locations. Before 2015 there were four families of engineered nucleases that were used by scientist namely; meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), and CRISPR/Cas9

system. The concept of genome editing is based on DNA double stranded break (DSB) repair mechanics. There are actually two pathways that repair DSB; non-homologous end joining (NHEJ) and homology directed repair (HDR). in NHEJ a variety of enzymes are used to directly join the DNA ends while in the HDR; a homologous sequence is used as a template for regeneration of missing DNA sequences at the break point this pathway is consider more accurate than the former. This can be exploited by creating a vector with the desired genetic elements within a sequence that is homologous to the flanking sequences of a DSB (6, 7).

The most critical step in genome editing is creating a DSB at a specific point within the genome. The Commonly used restriction enzymes are effective at cutting DNA, but generally recognize and cut at multiple sites. The scientist have created site-specific DSB, three distinct classes of nucleases have been discovered and bioengineered. These are the Zinc finger nucleases (ZFNs), transcription-activator like effector nucleases (TALEN), meganucleases and the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system (8).



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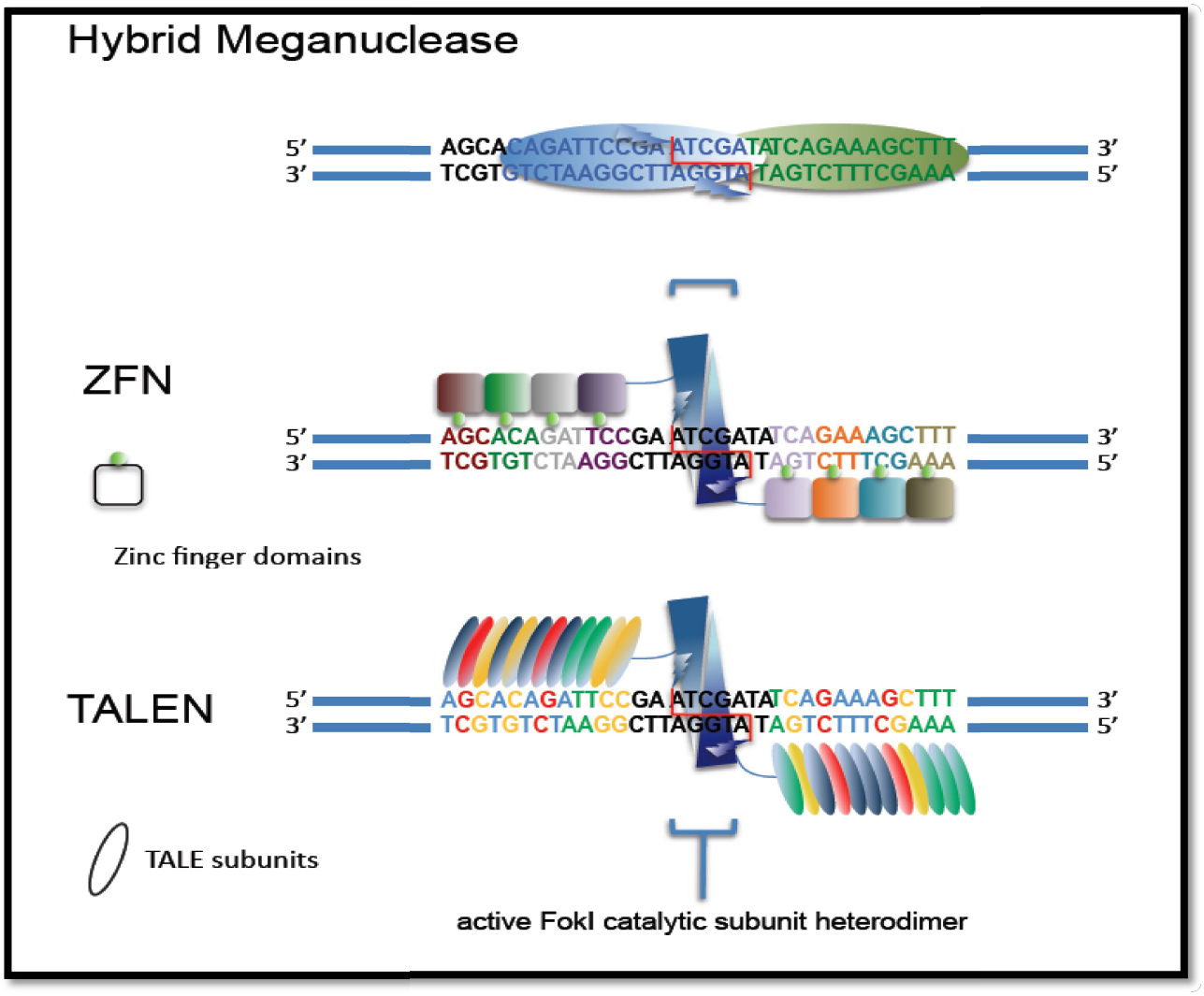
Meganucleases

Meganucleases was discovered in the late 1980s, they are enzymes in the endonuclease family characterized by their capacity to recognize and cut large DNA sequences from 14 to 40 base pairs. The well known meganucleases are the proteins in the LAGLIDADG family, which owe their name to a conserved amino acid sequence (9, 10). The Meganucleases are commonly found in the microbial species, they have unique property of having a long recognizable sequences greater than 14 base pair, this makes them naturally specific. The high throughput screening methods have been used to create meganuclease variants that recognizes unique sequence(11). Scientist have also altered the DNA interacting aminoacids of the meganuclease to design sequence specific meganuclease. Other approach involves the application of the computer models to predict as accurate as possible the activity of the modified meganuclease and the specificity of the recognized nucleic seaquence. A data bank containing several units of protein has been created, these units can be combined to obtain chimeric meganucleases that efficiently recognizes the target site.Meganucleases causes less toxicity in cells compared to methods such as Zinc finger nuclease (ZFN), this is because of more stringent DNA sequence recognition, and however, the construction of sequence-specific enzymes for all possible sequences is costly and time consuming.



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Zinc finger nucleases

Zinc finger nucleases (ZFNs) are a class of engineered DNA-binding proteins that facilitate targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations. The concept of ZFNs and TALEN techniques are based on a non-specific DNA cutting catalytic domain, which are then be linked to specific DNA sequence recognizing peptides such as zinc fingers and transcription activator-like effectors (TALEs).The initial step in ZFNs is to find an endonuclease whose DNA recognition site and cleaving site are separate from each other, this condition is not common among the restrictions enzymes. The cleaving portion is separated since it is non-specific thus no recognition potential, then linked to sequence recognizing peptides that could lead to very high specificity (12). Zinc-finger nucleases-induced double-strand breaks are subject to cellular DNA repair processes that lead to both targeted mutagenesis and targeted gene replacement at remarkably high frequencies (13).

ZFNs are artificial restriction enzymes generated by the process of fusing Zinc finger DNA binding domain to a DNA cleavage domain, this can be engineered to target specific DNA sequence of targets thus enabling ZFN to target unique sequence within a genome. The ZFNs consist of two protein domain; the DNA binding domain consisting of eukaryotic transcription factors and contain zinc finger, and the nuclease domain consisting of the FokI restriction enzyme which is responsible for catalytic cleavage of DNA (14).

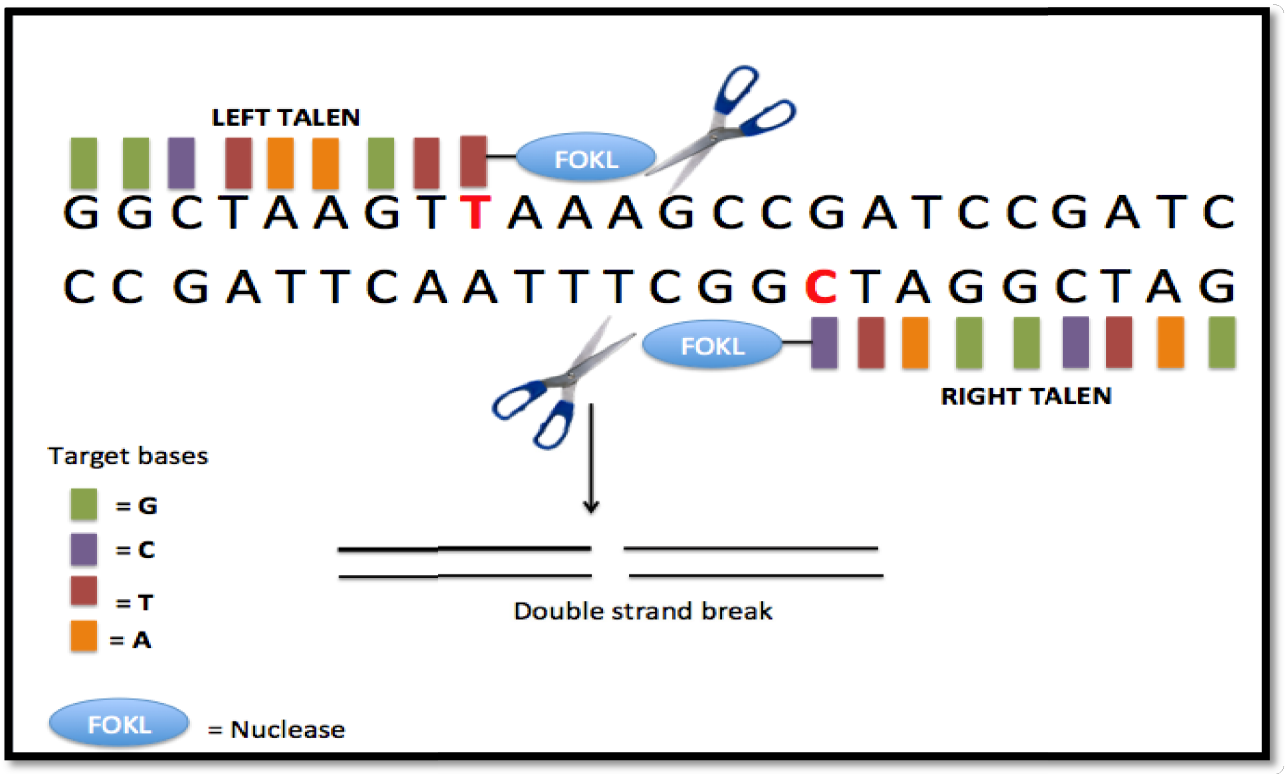
The ZFNs are important in the manipulation of the genomes of many plants and animals, they are also used to create a novel generation of genetic disease models known as the isogenic human disease models. They have also been used in a clinical trial of CD4+ human T-cells with the CCR5 gene disrupted by zinc finger nucleases to be save as a potential treatment for HIV/AIDS. The customized designed ZFNs combines the non-specific cleavage domain of FokI endonuclease with zinc finger protein offers a general way to deliver a site specific DSB to the genome hence stimulate

local homologous recombination by several orders of magnitude. The zinc finger domains are not specific enough for their target site, there are high possibility of off-target occurrence (14).

TALEN

The transcription activator like effector nucleases (TALENs) are specific DNA binding proteins that has an array of 33 or 34 amino acid repeats. They are artificially restriction enzymes developed by fusing the DNA cutting domain of a nuclease to TALE domains, which can be eventually tailored to specifically recognize a unique DNA sequence (13).Transcription activator-like effectors (TALEs) can be easily engineered to bind practically any desired DNA sequence. By combining such an engineered TALE with a DNA cleavage domain one can engineer restriction enzymes that will specifically cut any desired DNA sequence. When these restriction enzymes are introduced into cells, they can be used for gene editing or for genome editing in situ, a technique known as genome editing with engineered nucleases. The relationship between amino acid sequence and DNA recognition of the TALE binding domain enable efficient engineering of proteins. TALEN constructs are assembled, then inserted into plasmids; the target cells are then transfected with the plasmids, and the gene products are expressed and enter the nucleus to access the genome. TALEN constructs are delivered to the cells as mRNAs, this removes possibility of genomic integration of the TALEN-expressing protein. Using an mRNA vector can also dramatically increase the level of homology directed repair (HDR) and the success of introgression during gene editing (15, 16).

TALEN are used by scientist to edit genomes by inducing double-strand breaks (DSB), which cells respond to with repair mechanisms. This repair mechanism induces errors in the genome via insertion or deletion, or chromosomal rearrangement; any such errors may render the gene products coded at that location non-functional. It should be monitored when designing new systems. Alternatively, DNA can be introduced into a genome through NHEJ in the presence of exogenous double-stranded DNA fragments. Homology directed repair can also introduce foreign DNA at the DSB as the transfected double-stranded sequences are used as templates for the repair enzymes (15).TALEN technology has been used for instance to efficiently engineer stably modified human embryonic stem cell and induced pluripotent stem cell (IPSCs) clones and human erythroid cell lines. The technology has been used to experimentally correct the genetic errors that underlie disease. For example, it has been used in vitro to correct the genetic defects that cause disorders such as sickle cell disease, xerodermapigmentosum, and epidermolysisbullosa. It was also shown that TALEN technology can be used as tools to harness the immune system to fight cancers. In theory, the genome-wide specificity of engineered TALEN fusions allows for correction of errors at individual genetic loci via homology-directed repair from a correct exogenous template (17).



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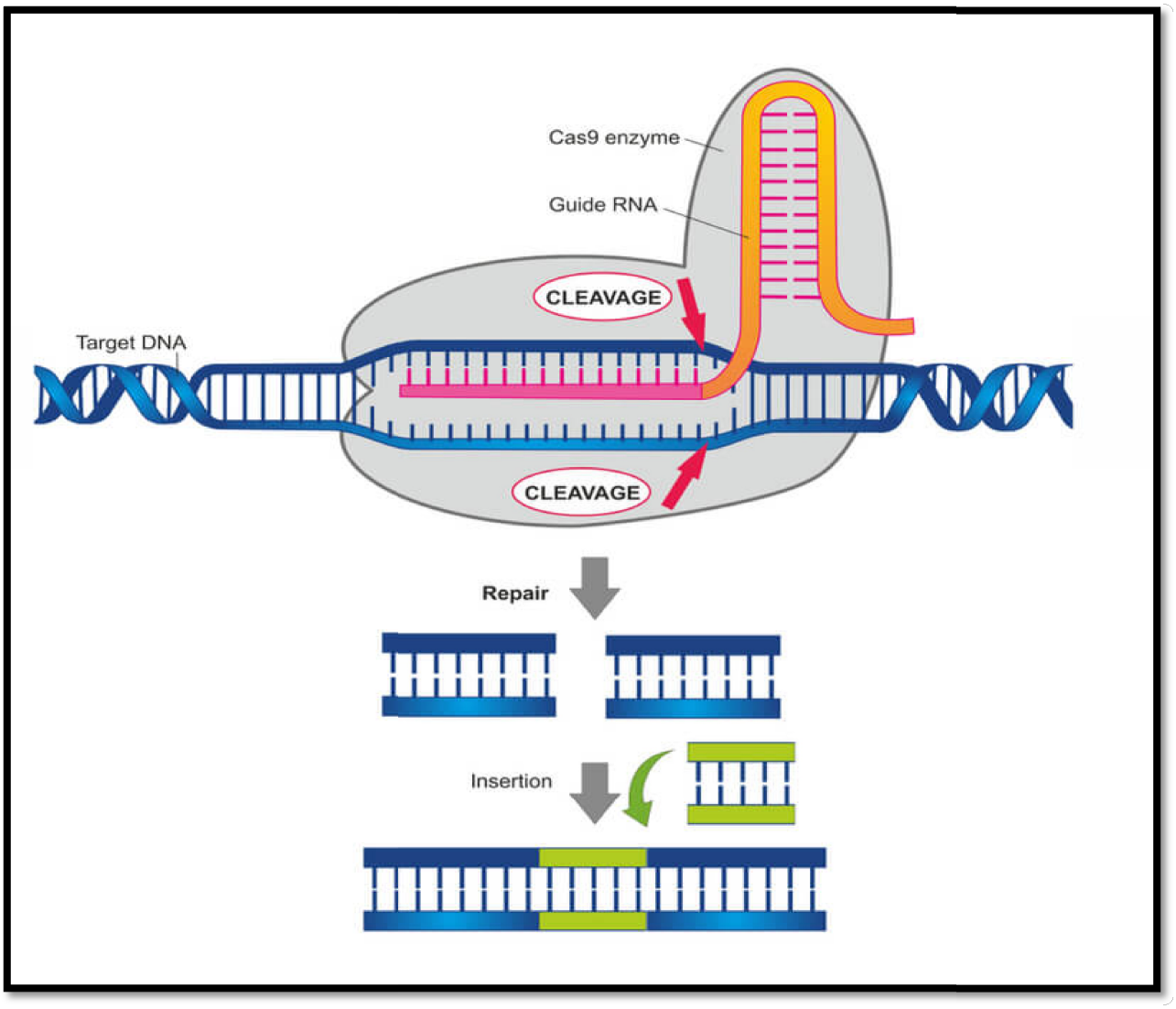
CRISPR and Gene Drives

The CRISPRs are Clustered Regularly Interspaced Short Palindromic Repeats; this are genetic elements that bacteria use as a kind of acquired immunity to protect against viruses. It consist of short sequences that originate from viral genome incorporated into bacterial genome. Cas (CRISPR associated proteins) process sequences and cut matching viral DNA sequences. By introducing plasmids containing Cas genes and specifically constructed CRISPRs into eukaryotic cells, the eukaryotic genome can be cut at any desired position (18).



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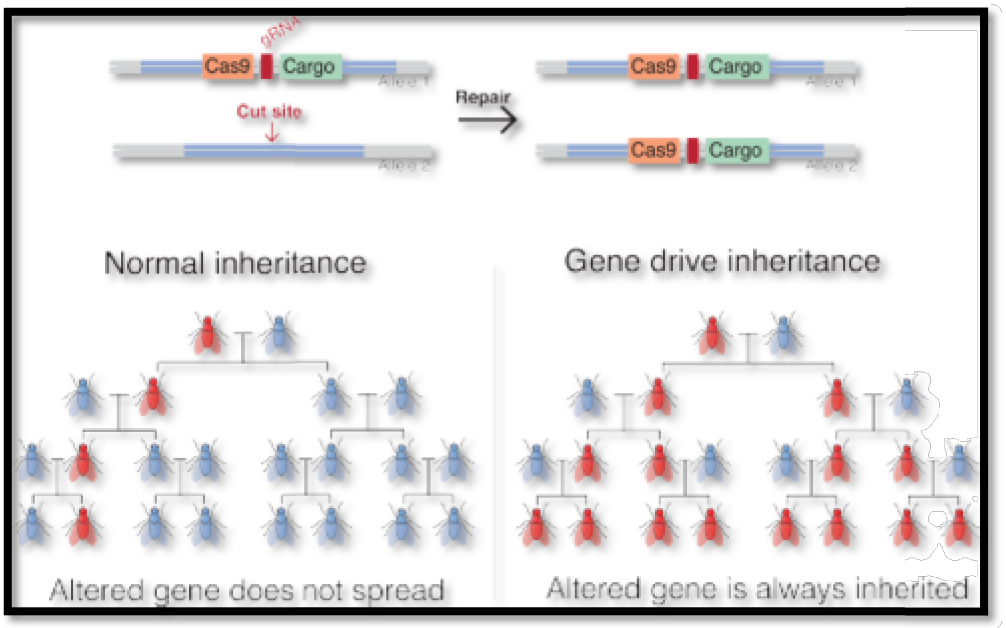
Gene-drive is a method that efficiently enable DNA sequence to be inherited by all organisms' offspring's, this is in contrast to mendelian inheritance, in gene- drive, a genetic element on one chromosomal copy duplicate itself onto the other chromosomal copy as a result the sequence is passed on 100% of the time to the next generation. This may eventually sweep the entire population of the species in an ecosystem. There is a growing interest in gene drive research especially with the advent of the CRISPR technology which enable placing DNA sequence that encode for the components of CRISPR system directly and precisely into its target site in the genome (19).

The CRISPR system can actually act as a gene drive and catalyze its own incorporation into each new chromosomal copy a good example is the CRISPR gene drives for controlling the population of disease-carrying insects, such as the mosquitoes that act as vectors for malaria or Dengue fever. In this theoretical scenario, a mosquito would be engineered with a CRISPR gene drive that is placed into, and so disrupts, a gene controlling the insect's ability to carry the parasite. This mosquito would then be released into the environment and allowed to mate with wild mosquitoes. As the gene drive sweeps through the population, the number of mosquitoes that can carry the parasite would drastically decrease. While using such a gene drive strategy to control insect-borne diseases has

significant potential for public health benefits, it is an environment-altering intervention where the ecological consequences are impossible to know with absolute certainty. Scientists and other stakeholders have called for further deliberation on whether or how such research and application should proceed (19).



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We can define gene-drive as the systems that are Mendelian inheritance biased, since they enhance the likelihood for a sequence of DNA passes between generations through sexual reproduction and potentially throughout the population.

CRISPR-Cas9 is a tool that enable genome editing or alterations that provides a molecular tool for altering regions of DNA in ways that could yield a gene drive. As a gene editing method it is easier to use, faster to develop, and more precise than techniques such as zinc finger nucleases and TALENs (20,22).

DNA Nanotechnology

DNA is hereditary material with simple and stable building blocks of phosphate- 2'deoxy ribose-organic nitrogenous base with unique 3-D structure with AT-GC paring of two anti-parallel strands. DNA in solid or solution is a good carrier of electrons and biocompatible with binding efficiency to many proteins, organic molecules and metal ions. With the introduction of nanotechnology in silver-gold nanoparticles and carbon nanoparticles, DNA has now been utilized as good source of nanotechnology material. DNA molecules have many interesting chemical properties – they are strands of chemical bases, with easily programmable sequences, that automatically and specifically bind to complementary sequences and form rigid double helices. These properties have caught the attention of scientists and engineers who see potential for DNA as an engineering material for data storage, computation and robotics (23).

Researchers have also used DNA as a material to build "nano-bots" that may one day be used, for example, to target drug delivery to specific cells. Because the shape of a DNA molecule can be modulated using small molecules or proteins, such as those on the surface of a drug target cell, the DNA can act as computational "logic gates" to control the opening and delivery of the drug. The field of DNA nanotechnology takes this molecule out of its biological context and uses its information to develop structural motif then bind them together. This field has had a remarkable impact on nano-science and nanotechnology, and has been revolutionary in our ability to control molecular self-assembly.

III. CONCLUSION

The emergence of technologies such as the genome editing technology that allow for genetic modification had made many countries around the world to grapple with how to regulate these technologies to ensure they can benefit society in the safest and fairest way. While different political and cultural contexts shape how different nations approach these issues, understanding the rationales behind each country's regulations can be instructive.

AUTHOR DECLARATION

The Author here by declares that there is no conflict of interest entailed in the production of this article.

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