The Analytical Review on Futuristic use of CRISPR-Cpf1 Aided Gene Drive Technology

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ABSTRACT

Organisms edited by gene editing or traditional selective breeding are typically less able to survive and reproduce which effectively prevents those alterations from spreading in the wild populations. This paper focuses on how the CRISPR-Cpf1 system can be used to build a Gene Drive capable of spreading particular alterations in the wild population, and its potential applications. Named for the ability to "drive" themselves and nearby genes through populations of organisms over many generations. Normally, the sexually reproducing organism comprises of 50-50% genetic information from both parents. But with gene drive, you can have 100% chance of passing a particular gene.

The discovery of new Gene Editing Technology, based on the bacterial immune system, allow us to edit genome at specific sites with more precision, accuracy and ease. CRISPR Gene Drives including the edited version of the targeted gene and additional sequences with the DNA cutting Cpf1 protein and Guide RNA gene. When an organism containing a Gene Drive, mates with the wild counterparts, the offspring inherit one altered and one original copy of the target gene. The Guide RNAs Cpf1 directs to cut the original copy which is repaired by copying the altered gene as template synthesizing Gene Drive sequence in its place. Because the organism now has two identical copies of the alteration and the Gene Drive one on each chromosome, all of the organism will inherit both components. The same process will be repeated in subsequent generation causing the altered gene and Gene Drive to spread into the entire wild population. The Gene Drive technology has the potential to save millions of lives and give us unprecedented control over the natural world. This technology can be used to eradicate insect-born diseases, empower sustainable agriculture and promote ecological conservation.

Keywords: CRISPR, Gene Drive, Guide RNAs, Cpf1 protein

Introduction

Anthony James was the pioneer to come up with a unique idea of making mosquitoes that didn't transmit malaria two decades ago. Given to the paucity of technology, the idea failed to be realised at that time. The task to make a laboratory malaria-resistant mosquito was nearly impossible. With the breakthrough of 2012, Clustered Regulatory Interspaced Short Palindromic Repeats, CRISPR-associated protein 9 (Cas9), or CRISPER-Cas9 gene editing revolutionary technology,[7] in short, Anthony A. James and colleagues at the University of California Irvine established a way to splice in a gene that aids the mosquito to produce and pour out antibodies to the parasite, making it almost impossible for the malaria parasite to survive inside the mosquito.
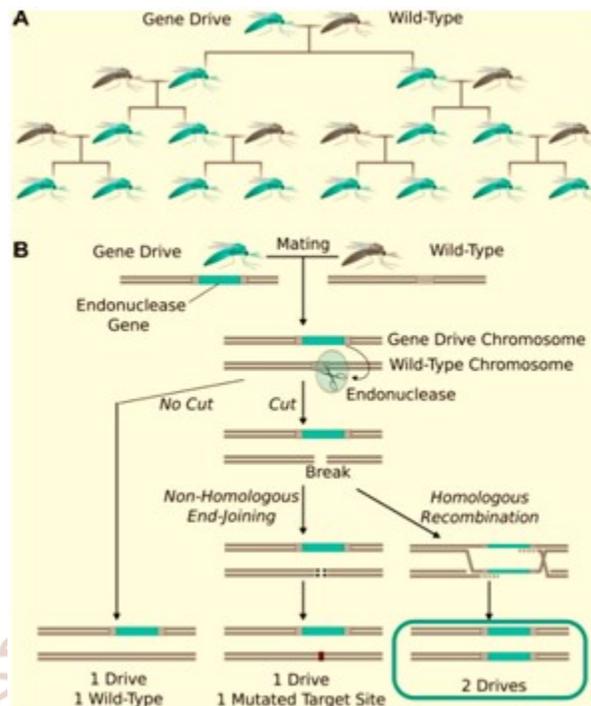
Figure 1: A: Gene Drives facilitating the transfer of a trait to all the progeny B: The underlying mechanism of trait inheritance via Gene Drives (Wyss Institute at Harvard University)
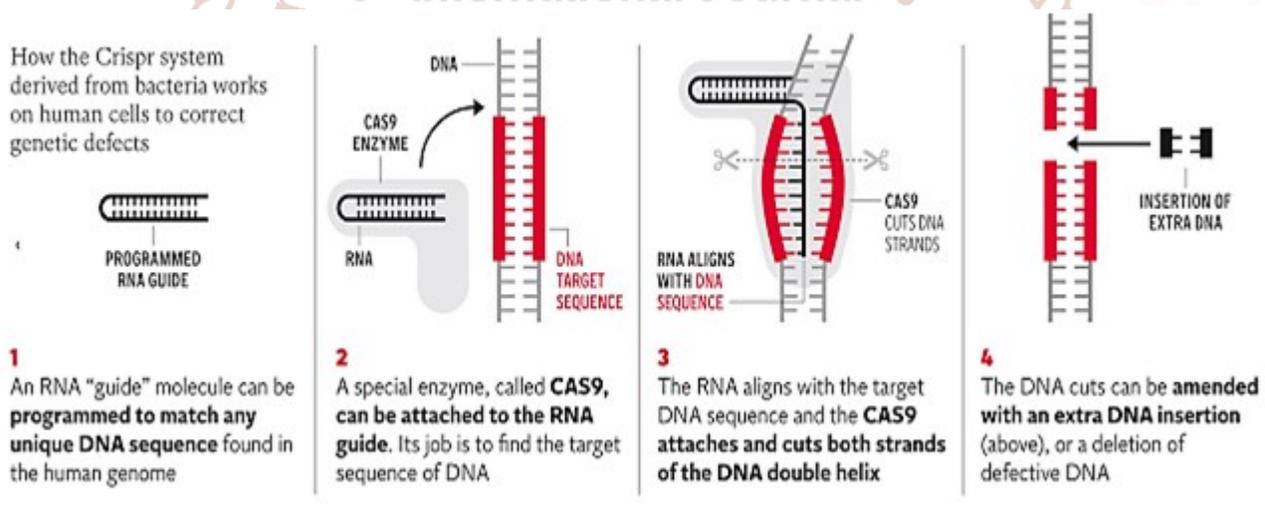

Figure 1 The schematic representation of CRISPR-Cas9 system functioning in a cell and insertion of foreign Gene

The major conundrum was to propagate the altered gene throughout the wild population. A possible way to do that could be to breed up some new genetically-engineered mosquitoes and release them into the wild population. It could be anticipated that mosquitoes pass on the genes to the progeny. The problem with this approach was that we have to release nearly 10 times the number of native mosquitoes to work. For instance, in a village with 10,000 mosquitoes, an extra 1,00,000 mosquitoes are required to be released[5].

While James relied on cutting-edge gene editing technology CRISPR-Cas9, Valentino Gantz and Ethan Bier from the University of California San Diego found a way to perpetuate that resistance to future generations of mosquitoes. An experiment was set up to test if the Bier's Gene Drive worked. They engineered two mosquitoes to carry the anti-malaria gene and also the new tool, a gene drive. Finally, they set it up so that any mosquitoes that had inherited the anti-malaria gene wouldn't have the usual white eyes,

but would instead have red eyes. This was to identify the mosquitoes that carried analtered gene from those carrying the non-altered gene (Wild-type).[3]

So two anti-malarial mosquitoes were taken, red-eyed mosquitoes and they were put in a box with 30 ordinary white-eyed ones and were left to breed. In two generations, they produced around 3,800 progenies.The experiment saw exciting results. They started with just two red-eyed mosquitoes and 30 white-eyed ones. The white-eyed descendants were expected according to the Law of Inheritance. However, all 3,800 mosquitoes had red eyes.[3]

The extraordinary findings by Anthony James turned out to be in compliance with the Bier's proposition. But getting only red-eyed mosquitoes violates a rule that is the absolute cornerstone of biology; [3] According to Mendelian genetics,when a male and a female copulate, their progeny inherits half of its DNA from each parent. So if the original mosquito was 'aa' and our new mosquito is 'aB', where B is the anti-malarial gene, the progeny should come out in four permutations: 'aa', 'aB', 'aa', 'Ba'. Instead, with the new gene drive,they all came out 'aB'. Biologically, that shouldn't even be possible.

Explanation: The above-mentioned results could be answered as follows. Firstly, AnthonyJames used CRISPR as a tool, a tool that allows researchers to edit genes very precisely, easily and quickly. It does this by harnessing a mechanism that already existed in bacteria. Basically, there's a protein that acts like scissors and cuts the DNA, and there's an RNA (guide RNA) molecule that directs the scissors to any point on the genome you want. The result is basically a word processor for genes. One can take an entire gene out, put one in, or even edit just a single letter within a gene. And one can do it in nearly any species. Thus, with CRISPER technology one could engineer a mosquito to be malaria-resistant, which was difficult 5-7 years ago.[3]

Additionally, a biologist at Harvard named Kevin Esvelt hypothesised that one can make it possible if CRISPR inserts not only your new gene but also the machinery that does the cutting and pasting. In other words, if CRISPR also copied and pasted itself this would set off a perpetual motion machine for gene editing. Esvelt created a CRISPR gene drive that not only guaranteed that a trait will get passed on, but if it's used in the germ line cells, it will automatically copy and paste a new gene into both chromosomes of every single individual. It's like a global search and replaces, or in scientific terms, it makes a heterozygous trait homozygous.[5]

II: Gene Drive and Cpf1

A new genetic tool besides Cas9 nuclease that so far has been democratising genetic engineering field and it is Cpf1 (Centromere and Promoter Factor 1), a new CRISPR nuclease to the applied biological material.[1] First characterised in 2015 by the Zhang Lab at MIT, Cpf1 was picked out of hundreds of potential CRISPR system studied by various bacterial species. It's sleeker, simpler and more versatile nuclease than Cas9 and is far better than the Cas9 nuclease in many ways.[10][8][1]

Cpf1 doesn't require tracrRNA, it requires only CRISPR RNA, reducing the size of the engineered cRNA (that require nearly 42 nucleotides)molecule required by half as compared to Cas9 (that required tracrRNA nearly 100 nucleotide cRNA), making genome editing cheaper.[6][8]

Cpf1(~3.8 Kb) is also smaller than Cas9 (~4.1Kb), which precisely is of importance when it comes to gene delivery. Combined with its shorter gRNA, Cpf1 is even easierto shuttle into cells via low capacity vectors such as the adeno-associated virus.[2] [6]

While Cas9 generates blunt ends after cutting, Cpf1 generates clips DNA producing sticky ends that are easy to work with. Researchers can design DNA inserts that dock perfectly on these overhangs, optimizing the Non-Homologous End Joining (NHEJ)repair pathway for DNA insertion. This means with only one pair of scissors Cpf1 can get even a cut above Cas9 in non-dividing cell types that rely mostly on the NHEJ repair mechanism.[11][2]

Cpf1 has an advantage over Cas9 in the sense that it cuts DNA ~18-23 bp downstream from the T rich PAM site,then G rich PAM (Protospacer adjacent motif) site.

"Protospacer adjacent motif (PAM) is a 2-6 base pair DNA sequence. In the CRISPR bacterial adaptive immune system, PAMoccurs immediately following the DNA sequence targeted by the Cas9 nuclease. PAM is a component of the breeching virus or plasmid but is not a component of the bacterial CRISPR locus. If Cas9 is not followed by

the PAM sequence, it will not successfully bind to or cleave the target DNA sequence". [15]

PAM is an essential targeting component (not found in thebacterial genome) which distinguishes bacterial self from non-self DNA, thereby preventing the CRISPR locus from being targeted and destroyed by anuclease. This means that cutting doesn't disrupt the PAM site allowing for multiple rounds of DNA cleavage that promises to increase opportunities for the desired genomic editing to occur. This is in comparison to Cas9 which cuts closer to its G rich PAM site resulting in Indels (Insertion deletion mutations) that destroy the recognition sequence and prevent further rounds of cutting.[1] [15]

Zhang and his team at MIT have already identified two Cpf enzymes, out of 16 Cpf1-family proteins, fromAcidaminococcus and Lachnospiraceae, that can carry out efficient genome-editing activity in human cells opening up new avenues for research and therapeutic applications.[11][1][8]

A gene drive is a technique that guarantees that a specific gene can be edited. They work by biasing the inheritance of genes. Normally a sexually reproducing organism comprises its genome50-50 from the genetic information of its parents. Gene drives changes this. In a literal sense, a gene drive is a mechanism such as CRISPR attached to an organism's chromosomes. And it biases the way those genes are passed along. Instead of there being a 50% chance of passing along a marker gene, one can have a near 100% chance of passing it down to offspring and that offspring will also have nearly 100% chance of passing it on to its offspring. In other words, gene drives can break rules of Natural Selection and Mendel's Law of Inheritance.[10]

To ensure that scientist can safely study gene drives in the laboratory Wyss Institute scientist have developed proactive safeguards to prevent them from accidentally spreading in the wild causing unintended side effects. The first safeguard is **Split Gene Drive** in which two components of the gene drive are split and only one component of the gene drive are included in the altered gene. For eg: An organism carrying a split gene drive might have the altered gene together with the sequence encoding guide RNAs. When it meets with the laboratory organism carrying the Cas9/Cpf1 gene, the drive is active causing the alteration and guide RNAs to be inherited by all offspring. But when it meets with the wild

counterparts that are not carrying the Cas9 gene, the gene drive does not function. The second safeguard is to insert an **Artificial Target Sequence** in the target gene of the laboratory organism. The gene drive is then constructed using guide RNAs that direct Cas9 cut only the artificial sequences. So it only works in the engineered laboratory organism but not the wild population.[11]

As an additional safeguard, the team has developed a way to undo an alteration created by an earlier gene drive. To do this, they would first need to initiate a gene drive. If later on, they wanted to reverse the imposed genetic changes, they would apply another gene drive to cut out a unique gene sequence found in the first gene drive. This would then reverse the original genetic alteration using the latter gene drive to eliminate the former gene drive across the population. The team recommend that all laboratories conducting gene drive experiments incorporate at least one of the described safeguards. Together, these advances offer a way to safely explore the potential of CRISPR gene drive technology. [11] [12]

Three genes namely (AGAP005958, AGAP011377 and AGAP007280), so far have been identified that confer a recessive female sterility phenotype upon disruption, and when inserted into each locus, CRISPR-Cas9 gene-drive constructs designed to target and edit each gene. For each locus targeted, thestrong gene drive at the molecular level has been observed, with transmission rates to the progeny of 91 to 99.6%. Population modelling and cage experiments indicate that a CRISPR-Cas9/Cpf1 construct targeting one of these loci, AGAP007280, meeting the minimum requirement for a gene drive targeting female reproduction in an insect population. [2]

III: Potential applications and pros &cons

The current scenario projects that that gene drives didn't work very well and still in the stage of development. With the evolutionary point of view tinkering around with an organism's genes makes them less evolutionarily fit. Therefore, biologists can make all the mutant fruit flies required and if some escape, natural selection just takes care of them.

The remarkable, powerful and frightening fact about gene drives is that that will no longer be true. Assuming that your trait does not have a big evolutionary handicap, like a mosquito that can't fly[2], the CRISPR-based gene drive will spread the change relentlessly until it is in every single

individual in the population. [11] Now, it isn't easy to make a gene drive that works that well, but James and Esvelt think that we can. According to a report of WHO in 2015, there were 214 million cases of malaria 4, 38,000 people died of the disease.[8]

The gene drives open avenues to some exciting and remarkable possibilities. If an anti-malarial gene drive is inserted in just 1% of Anopheles mosquitoes (the species that transmits malaria), estimate suggests that approximately within a year, it would spread to the entire population thereby virtually eradicating malaria in a year. But it still stands as a hypothesis while the unprecedented repercussions are yet to be evaluated. A thousand children a day die of malaria. In a year, that number could be almost zero. The same goes for dengue fever, chikungunya, yellow fever.[9]

Other possible applications of gene drives are the prevention of endangered species from becoming extinct. For instance, the Asian carp is an invasive species of the Great Lakes and is desired to be removed. The possibility could be to release a gene drive that makes the fish produce only male offspring. This will result in a few generations, no females left, no more carp. In theory, this means we could restore hundreds of native species that have been pushed to the brink.[3]

Gene drives are efficacious enough to change an entire species if released accidentally and often very quickly. Anthony James, for instance, took precautions as he bred his mosquitoes in a bio-containment lab and also used a species that's not native to the US so that the accidental escape of mosquitoes will not have dire consequences since there'd be nothing for them to mate with and they'd die off eventually.

Alongside, it's also true that the native Asian carp population will disappear provided a dozen Asian carp with the all-male gene drive accidentally got carried from the Great Lakes back to Asia.[9] And that's not so unlikely, given how connected our world is. In fact, it's why we have an invasive species problem. And that's fish. Mosquitoes, fruit flies, rodents are difficult to be contained within a particular region. They cross borders and oceans all the time.[11] [12] [13]

The other possibility lies that a gene drive might not stay confined to the target species. That's because of a phenomenon like agene flow in which the

neighbouring species sometimes interbreed. In that case, it's possible a gene drive could cross over like Asian carp could infect some other kind of carp. If the gene drive just promotes a trait, like an eye colour, the negative consequence can be avoided. However, there's a decent chance to see a wave of avaried variety of fruit flies in the near future. But it could be a disaster if the drive is designed to eliminate the species entirely.

The last conundrum is that the technology to genetically engineer an organism and include a gene driveis basic enough to be reproduced in any lab around the world whether it should be democratised or contained within labs.[11][14]

Gene drives also have some limitations. They work only in sexually reproducing species. Thus they can't be used to engineer viruses or bacteria. Also since the trait are inherited with each successive generation only. Therefore, changing or eliminating a population is practical only if that species has a fast reproductive cycle, like insects or maybe small vertebrates like mice or fish. In elephants or people, it would take centuries for a trait to spread widely enough to matter.[3] [13]

The misuse of Gene Drives, even with CRISPR, is unrealistic.[5] To engineer a truly devastating trait, for instance, to make a fruit fly that feeds on ordinary fruit instead of rotting fruit with the aim of sabotaging American agriculture, initially the genes controlling fly instincts to eat fresh besides rotten fruits need to be worked out. Then alteration of those genes to change the fly's behaviour is altogether another challenge. And since there is an on-allelic interaction that controls a single character of an organism, this further dilutes the possibility. Theoretically, it is easy to build what's called a reversal drive that basically overwrites the change made by the first gene drive. So if the traits aren't transferred in the desired form, the second drive with antagonistic effects could cancel out the mutation, at least in theory. [11]

Conclusion

Gene drives have the ability to change entire species at will. But the ethical questions arises whether we should or we should not engineer species carrying such drives. Moreover, the standards are to be laid in order to regulate the practice to engineer such GMO's. With the technological point of view, the possibilities are numerous but are cornered and favour only fast reproducing organisms. The regulation of Gene Drives is highly debatable. At the same time,

this technology still requires a conversation. And given the nature of gene drives, that conversation has to be global. What if one country wants to use it and other doesn't? Who decides whether to release a gene drive that can fly? are some pending questions that need to be discussed widely.

Both public and scientist should talk honestly about the risks and benefits and should take accountability for the choices that are finally made. Not just the choice to use a gene drive, but also the choice not to use one. Gene drives possess some risks, and those risk should be addressed via open dialogue and colloquium. But at present, malaria exists and kills 1,000 people a day[8]. And to combat it, spraying pesticides does grave damage to other species, including amphibians and birds and that's even worse.

Acknowledgement

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