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Genome Engineering Using the CRISPR Cas9 System

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Abstract

CRISPR technology is the most widely used genetic editing tool to edit, modify, delete, add and replace DNA sequences in both prokaryote and eukaryote, the normal CRISPR pathway cut gene at a specific point by recognizing a Protospacer Adjacent Motif (PAM) and induce double strand break, the cell can repair this destruction through Non-Homologous Enjoining. Scientist takes advantage of this break and inserts a homologous strand with specific changes through Homologous Directed Repair (HDR). CRISPR Interference (CRISPRi) follow the same technique but use a dead, mutated or inactive form Cas9 protein to render gene inactive and thereby block gene expression. In this study we compare both the similarities between CRISPR-Cas9 system and CRISPR Interference and further shown the differences of off-target in both systems.

Keywords: CRISPR; CRISPR Interference; Off-target; Prokaryote; Eukaryote

Introduction

Throughout the last century, scientists have been trying to find an efficient, reliable, simple and cheap technique that can be used to edit and modify both plant and animal genome [1]. The acronym CRISPR stands for Clustered Regularly Interspace Palindromic Repeat which is scientist potential weapon and tool for treating hundreds of genetic diseases such as Sickle Cell Anemia (SCA), Cystic Fibrosis (CF), Muscular Dystrophy (MD), Huntington disease etc. [2-4]. CRISPR system is originally discovered in Bacteria which serve as vaccination card and immune mechanism against invading viral attack e.g. bacteriophage [5,6].

Prior to the discovery of CRISPR, scientist relied on two gene editing techniques Zinc Finger Nucleases (ZFN) and Transcription Activator-Like Effector Nucleases (TALENS) [1,7]. ZFN was first used in 2002 to edit the genome of Drosophila melanogaster known as fruit fly and it application in human cell was first reported in 2008. TALENS was used in 2010 to edit genome of yeast and later on human cells [1,3,4,8]. Adeno-Associated Virus (AAV) is another genetic tool which incorporates engineered restriction enzymes along with a form of Recombinant Adeno-Associated Virus (RAAVs), this virus is non-pathogenic in nature and also present in all stages of mammalian cell cycle. The system took advantage of the integration of the virus into the host DNA at probable site and thus, can be modified to edit specific gene in a genome. Just like TALENs and ZFN, AAV has Its own limitations as production of vectors are extremely difficult and has low capacity to accommodate high amount of genetic content [6,8] (Table 1).

Evolution of CRISPR

The discovery of CRISPR is revolutionary and full of promises as it offers high efficiency, very simple to use and cheap compare to both ZFN and TALENS. CRISPR was first discovered in 1987 by group of Japanese scientist led by Ishino who found a unique DNA sequence in *Escheria coli*, these sequences are palindromic in a nature (i.e. they appear or read identically both forward and backward e.g. ATCTA) but they could not ascertain or describe it function. In 1990, Francisco Mojica found similar sequences in an *Archaea* which are microorganism that resemble bacteria but contain different genetic heritage [3,5,9,10]. He extracted the sequences and inserted it into Basic Local Alignment Search Tool "BLAST" for DNA and the result shown the sequence to be of a viral DNA [10] (Figure 1).

The turning point of CRISPR came as a result of research by Barrangou and Horvath who were working in a yoghurt company, they

Gene Editing Tool	Type of Endonuclease	Length of Target site	Off-target rate	Rate of Mutation	Application
Zinc Finger Nuclease (ZFN)	FokI	18-36	High	10	Nematode, Tobacco, Zebrafish, Mice, Pigs and Human cells.
Transcription activator-like effector nucleases (TALENS)	FokI	30-40	Low	20	Water flea, Mice, Cow and Human cells.
Clustered Regularly Interspace Palindromic Repeat (CRISPR-Cas)	Cas9	22	Variable	20	Drosophila, Wheat, Maize, Rice, Monkeys and Human cells.

Table 1: Comparison of ZFN, TALENs and CRISPR-Cas system [6].

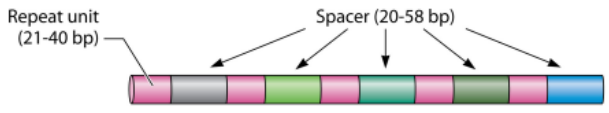

Figure 1: CRISPR locus (array) [9].

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discovered that the bacteria use to process the yoghurt are mostly killed during process leading to low production. They extracted the cultures and found out some strains are resistant to viral attack, they introduced the CRISPR array into bacteria that were susceptible and in turned they became resistant [3,4]. This discovery paved a way for scientist and shown experimentally that CRISPR is an adaptive system against viruses. They also discovered an important gene next to the CRISPR locust which code for a protein known as Cas9, an endonucleases which main function is to cut viral DNA [5,10].

The final recipe of CRISPR came as a result of research by Emmanuelle Charpenteir who discovered a specific RNA known as trans-activating or tracrRNA which contribute to the maturation of precursor RNA to CRISPR RNA (CrRNA) and a joint research by Charpenteir and Jenifer Doudna to create an artificial single guide RNA (SgRNA), a chimeric form which contain both TracrRNA and CrRNA. The first application of CRISPR to eukaryotic cell is done by Feng Zeng and Gorge Church who employed CRISPR technology to edit mammalian cells [4,10].

The application of CRISPR boomed from 2014 till today, many applications are becoming evident. Chinese scientists have successfully cloned 5 gene edited macaque monkeys to induce symptoms of sleeping disorder. The work of Jiankui has shown the application of CRISPR to make human cells resistant to HIV viruses. Researchers have employed this technology to developed genetically modified pigs that are prevented from Classical Swine Fever Virus (CSFV). MIT and Harvard researchers led by Feng Zhang has developed several version of SHERLOCK and HOLMES diagnostic kit which can be used as a biosensor to detect Zika and dengue viruses [11].

The discovery of CRISPR has open a way to unlimited applications, scientist has employed these technique to precisely edit, remove, add and replace DNA sequence and knock in and out gene of interest of different organisms ranging from prokaryote to eukaryote. Scientists have used CRISPR technology to make some specific changes in human embryos, monkeys and mice. It is also use to eliminate the DNA of integrated HIV viruses for infected human cells. Chinese scientist employed CRISPR to turn off genes in cancer patient [8]. It use is also reported to clone five gene of edited macaque monkeys to induce symptoms of sleeping disorders. Researchers have developed Genetically Modified Pigs (GMP) that are immune to Classical Swine Fever (CSFV) [9,10].

CRISPR mechanism in prokaryote

In prokaryote mostly Bacteria and *Archaea*, CRISPR provide a defence mechanism against bacteriophage. When the DNA of bacteriophage entered into Bacterial cell, it replicates inside and thus burst out by breaking or destroying bacterial cell wall which lead to the death of the bacteria. To prevent this type of destruction, bacteria overtime evolve an immune mechanism known as CRISPR which detect viral DNA, clipped it and destroy it. This system functions through three steps [9,12].

The first step known as adaptation stage occurs when a bacterial cell came in contact with a strain of viral DNA for the first time, the CRISPR array which is made up of sequence of repeat separated by a unique sequence known as spacers with Cas genes adjacent to the array along with Cas 1, Cas 2 and Cas 9 work together to locate the PAM sequence present in the viral DNA, cleave it and remove the PAM next to it and integrate the spacer in to the leader end of the CRISPR array [4,5,9,13].

The second stage which is the recognition stage occurs when the phage attack or inject it's DNA for the second time into the host cell (Bacteria or *Archaea*), the host CRISPR loci transcribed it spacers into small form of non-coding RNA known as Pre-CRISPR RNA which link up through base pairing with another RNA called Trans-activating (TracrRNA) to form a mature CRISPR RNA CrRNA [5,13]. The Cas gene adjacent to the loci is translated into a Cas9 protein which host the CrRNA and if the sequence of the CrRNA matches the viral DNA, it will cleaved it and thus destroy the phage DNA thereby preventing the viral DNA from replicating into and destroying the host cell. The difference between the viral DNA and the one stored in the host CRISPR loci is the presence of the PAM in the viral DNA known as "NGG" which is distinct with the "GTT" present in the CRISPR loci [5,9,12] (Figure 2).

CRISPR mechanism in eukaryote

The discovery of CRISPR-Cas9 system in both *Archaea* and Bacteria has open a way and led scientists to mimic the same technique and mechanism to introduce a deliberate and specific changes in the genome of eukaryotic cells. The CRISPR-Cas9 system which serves as an endonuclease employed a guide RNA which is the integration of CrRNA and TracrRNA and bind to the target through base pairing [8,14]. The cleavage of DNA along Cas9 and SgRNA occurs via two domains. Cas9 enzyme is known as an endonuclease enzyme that is mostly isolated from different bacterial species such as *Streptococcus pyogenes, Staphylococcus aureus, Brevibacillus laterosporus*, and *Streptococcus thermophilus*. Among all these bacteria, *Streptococcus*

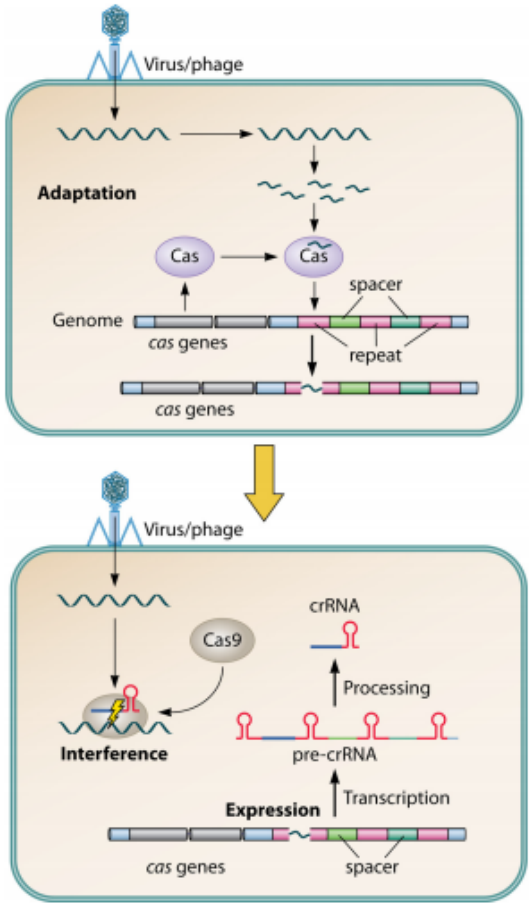

Figure 2: Stages of CRISPR-Cas mechanisms in prokaryote [5].

pyogenes is the most widely adopted bacteria for CRISPR-Cas9 system [3].

The two domain of Cas9 function differently, the opposite strand of the DNA is cleaved by RucV-domain while the HNH domain function by cutting the complementary strand of the CrRNA. Single guide RNA is a synthetic form of RNA which is 100 nucleotides in length; the first 20 nucleotide sequence in the 5' location drives the cas9 to a specific cutting site [1] (Figure 3).

Genome Editing or genome engineering is a process that involves a deliberate modification of genome such as insertion (addition), deletion (removal) of DNA sequence. Genome engineering has a potential in almost every field of life sciences, it has a great potential in solving food crisis in the world by increasing crop yield, preventing crop disease and controlling pest. It has a wide array of potentials in industrial growth by incorporating Genetically Modified Organism (GMO) such as bacteria and yeast in the production of milk, cheese, bread, alcohol and in pharmaceutical companies [6] (Figure 4).

CRISPR has become the most important and reliable tool employ by scientist to edit and make changes in DNA at almost every locus [6]. CRISPR as a tool use programmable component known as guide RNA which serve as GPS to guide the Cas9 enzyme into the target side which is complementary to the SgRNA and bind through Base Pair (BP) upstream the PAM, the Cas9 endonuclease exert a Double Strand Break (DSB) on the gene [15] (Figure 5).

Once the DNA is cleaved, cell must find a way to repair the break and this is achieve through Non-Homologous Enjoining (NHEJ)

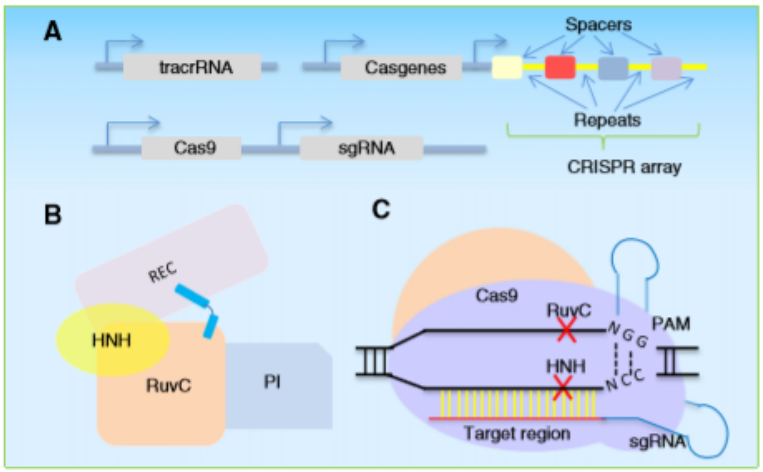

Figure 3: Components of type II CRISPR-Cas9 system [7].

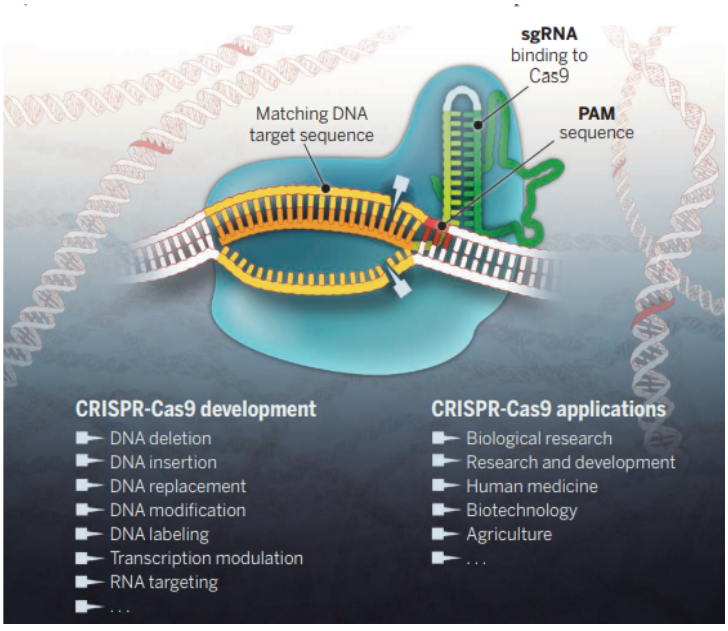

Figure 4: The use and application of CRISPR system [9].

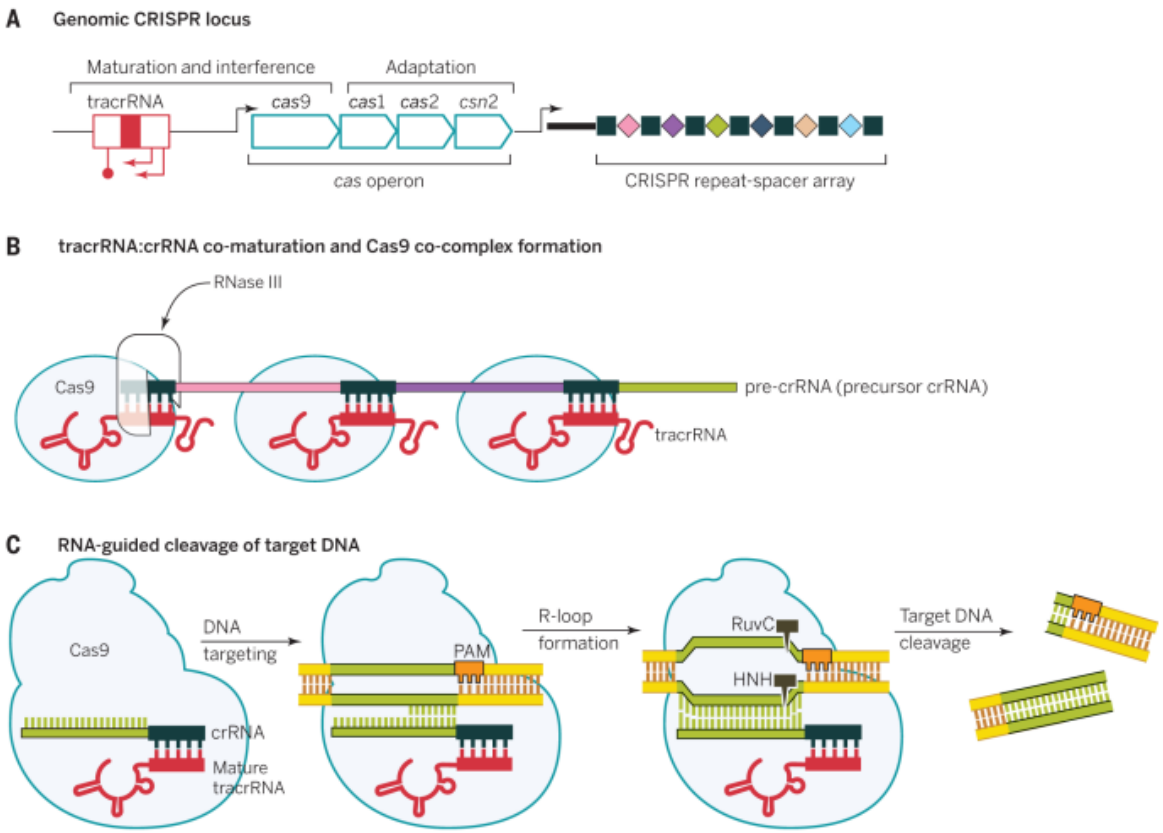

Figure 5: The development of engineered CRISPR/Ca9 systems [14].

and Homology Directed Repair (HDR) [16]. NHEJ is a simple repair mechanism where cells add or delete nucleotide known as indels, but this mechanism is highly prone to errors and can lead to frameshift mutation. NHEJ is employ by scientist with the aim to disrupt the function of the gene so that there will be no protein formation. HDR on the other hand, is the most efficient repair mechanism for the purpose of replacing a gene or DNA sequence with a newly synthesize sequence (i.e. gene insertion) [17]. This is accomplish by directly introducing a homologous donor which contain modification (insertion of nucleotide) and thus cell use the donor to repair the break [6,8,18-20] (Figure 6 and Table 2).

CRISPR interference (CRi)

The approach of silencing gene using CRISPR interference has added a greater dimension to genetic engineering and offer high potential in solving diseases associated with genetic disorders. It is simple to use and affordable [21]. CRi is a type of gene or DNA silencing technique which has shown to be an efficient technique for studying gene function in both prokaryotic and eukaryotic cells [22]. Recently CRi was employed to silence or study gene function in *Escheria coli*, mycobacteria, mammalian cells and many microbial cells [23].

It adopted the same mechanism as CRISPR-Cas9 system but uses a dead (mutated/inactive/dCas9) Cas9 protein instead of normal Cas9 system that has the ability to cleave and exert DSB on target sequence [24]. Dcas9 along with SgRNA survey the gene through the 20 base pair nucleotide and recognize the PAM sequence, bind to it and block the sequence from undergoing translation and gene expression [24] (Figure 7 and Table 3).

Application of CRISPR-Cas system to knockout disease markers

Disease associated with DNA: So many diseases occur as a result of genetic mutation, as a single point nucleotide mutation can disrupt gene function such as sickle cell anemia. Gene knockout method has been adopted by researcher to study genes that are responsible for many diseases [25]. CRISPR-mediated knockout screens have been employed in the identification of genes that are responsible for cancer progression and drug resistance [26]. Different studies have adopted Cas 9 system to knock out genetic mutation using patient-derived cells for Duchenne muscular dystrophy. Another significance CRISPR knockout method is the used of human immune cells along with CRISPR mediated knockout to make cells resistant to HIV infection [25].

Disease associated with RNA: DNA Genome is the same in all cells of organisms and doesn't change throughout lifetime. RNA carry instruction to turn stem cells to specific kind of cells like skin and brain cells, there are a lot of diseases where it is the RNA messages that goes wrong inside of our cells such as breast cancer, colon cancer, skin cancer, leukemia, Duchenne muscular dystrophy etc.. These types of disorders might respond better to RNA based solution. Recently Gooternberg et al., harnessed the collateral cleavage of C2C2 (Cas 13a) to detect RNA-Target hybridization using SHERLOCK (specific high efficiency enzymatic reporter unlocking). A collaborative research my MIT and

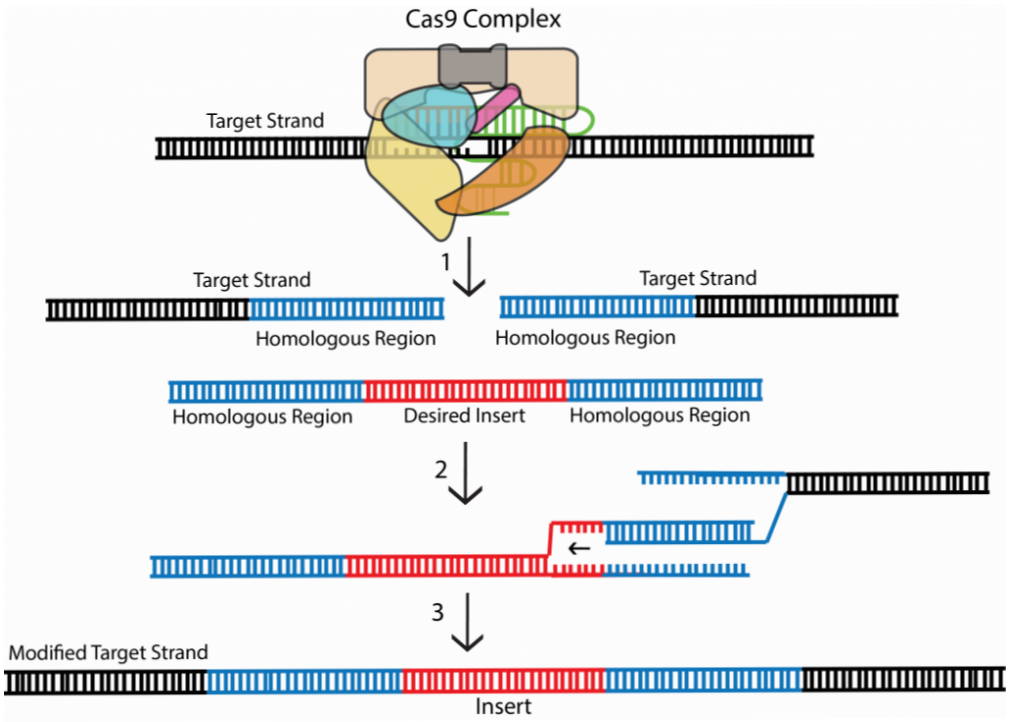

Figure 6: A representation of HDR mechanism [10].

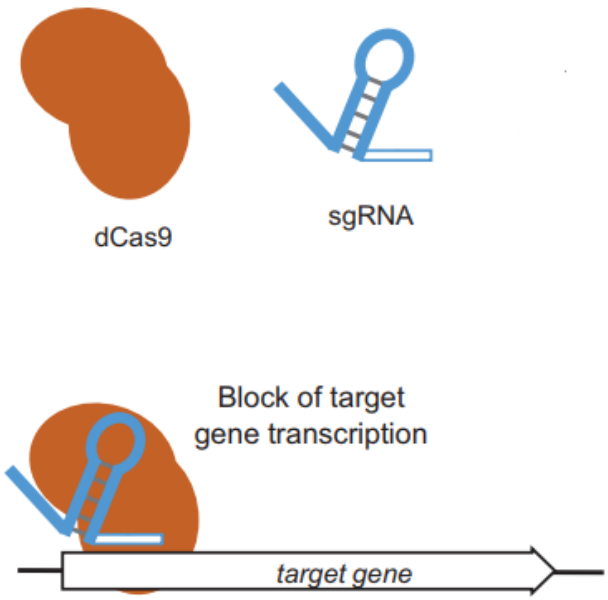

Figure 7: A representation of dCas9 and SgRNA (complex) and CRi between target gene and the complex [23].

Harvard led by Feng Zheng developed an upgrade of SHERLOCK which can detect Zika virus, Dengue Virus, Bacterial Isolate, Antibiotic resistant gene, Human DNA genotype, Cancer Mutation by amplifying RNA using isothermal amplification and using Cas 13 RNA activity foo cleavage and detection [26].

Prokaryote	Eukaryote
Occur natural.	Artificial using programmable Cas system.
Prokaryotes such as Bacteria and Archaea use CRISPR-Cas system as a form of adaptive system/defense mechanism against viral attack (e.g. Bacteriophage).	CRISPR-Cas system is employed by scientist to induce single strand or double strand break on eukaryotic genes to either silence the gene by binding (CRISPR Interference) or cleaved the gene and replace with another one through HDR or normal NHEJ.
Bacteria and Archaea use two different types of RNAs; a matured CrRNA which is the combination of Pre-CrRNA and tracrRNA which recognise viral DNA through PAM and bind or cleave to it.	It uses a single RNA known as guide RNA with 20 nucleotides specific to directing the RNA to a target through PAM sequence.
Cas 1 and Cas 2 are used in adaptive stage in which the Cas proteins cleave into a new viral DNA, remove it spacer and placed it in the host CRISPR array.	No adaptive stage.

Table 2: Difference between CRISPR in prokaryote and eukaryote [18-20].

Conventional CRISPR and CRISPR interference limitations

Off-target: Every technology comes with it on constraint, CRISPR as a gene editing tool is a promising technology that can help scientist counter genetic diseases, therapies and bring back extinct animals and species. Off-target effect is the number challenges of using this tool for gene therapy in human cells [27]. Due to diversity of genes in cells and similarities between genes, a target sequence may bind to undesired site which may lead to disruption of gene and it expression. Off target

CRISPR-Cas	CRISPRi
It is not reversible	It is reversible.
Easier to engineer	Much more easier to engineer.
It uses a normal (lively) Cas9 system to.	It uses a dead inactive/mutated (inactive/mutated) Cas9 system.
It induce double strand break on target gene.	It does not induce DSB on target but block gene expression.
It requires a Non-Homologous End-Joining (NHEJ) mechanism and requires a Homology-Directed Repair (HDR) mechanism or the presence of repair donor DNA templates.	There is no need for Non-Homologous End-Joining (NHEJ) mechanism and it does not require a Homology-Directed Repair (HDR) mechanism or the presence of repair donor DNA templates.

Table 3: Difference between CRISPR-Cas and CRISPRi [23].

effects are consequences of non-specific activity of the Cas Nuclease system in non-target locations [28].

This is also minimized when working using in vitro technique where cell lines carrying the desired genotype are pre-selected. Several studies employing CRISPR gene editing technique have reported that more than 50% of RNA-guided Cas system induced mutations. The system can tolerate between 2-5 mismatches, any number of mismatch greater than 5 can lead to dysfunction or result in different protein [29].

Just like normal CRISPR-Cas system, CRi can also affect neighbouring genes which may have similar sequences and thus it can result in an unexpected gene malfunction. It also has limitation on targeted genes due to its reliance on PAM (i.e. not all genes contain PAM sequences) and challenges in designing functional gRNAs [28].

Another challenge of using CRISPR Cas system is the introduction of nucleotide (i.e. indels) at a site of the target due to cells non homologous end-joining repair mechanism. Those indels have become a serious issue due to their participation in gene disruption, splicing stage and regulatory sequences for different application in which indels are unwanted or not desired [19].

Scientist have been trying to increase the specificity of target and the use of computational method such as Elevation developed by Listgarten, the use of convolutional and feedback Neural network to assign score on bases, to increase on target effect and reduce off-target. However the use of cpf1 as the Cas Nuclease has been promising in reducing off-target effects [30,31].

Conclusion

The emergence of CRISPR Cas system and CRISPR interference as a gene editing tool and regulation of gene expression has revolutionised medicine and therapy. Scientist work day in and day out to uncover it potentials in many applications ranging from correcting genetic diseases such as sickle cell anaemia, cancer therapy, HIV cure. The technique remains an option in botany and agriculture as a tool to improve crop quality and yield and to prevent plant disease and pest. It use in industry can lead to industrial growth and high production by employing genetically modified organisms such as bacteria and yeast in alcohol fermentation, bread production and beverages storage. With all these potentials, CRISPR still have some limitations and challenges with off-target as number one enemy where Single guide RNA mistakenly bind to another gene and knock it out or block the gene from translation into a protein.

References

1. Ma X, Zhang Q, Zhu Q, Liu W, Chen Y, et al. (2015) A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. Mol Plant 8: 1274-1284.
2. Cui L, Vigouroux A, Rousset F, Varet H, Khanna, V, et al. (2018) A CRISPRi screen in *E. coli* reveals sequence-specific toxicity of dCas9. Nature Communications 9: 1912.
3. Liu X, Wu S, Xu J, Sui C, Wei J (2017) Application of CRISPR/Cas9 in plant biology. Acta Pharmaceutica Sinica B. 7: 292-302.
4. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, et al. (2007) CRISPR provides acquired resistance against viruses in prokaryotes. Science 315: 1709-1712.
5. Bhaya D, Davison M, Barrangou R (2011) CRISPR-Cas systems in bacteria and archaea: Versatile small RNAs for adaptive defense and regulation. Annu Rev Genet 45: 273-297.
6. https://www.synthego.com/resources/crispr-101-ebook.
7. Song G, Jia M, Chen K, Kong X, Khattak B, et al. (2016) CRISPR/Cas9: A powerful tool for crop genome editing. Crop J 4: 75-82.
8. Hsu PD, Lander ES, Zhang F (2014) Development and applications of CRISPR-Cas9 for genome engineering. Cell 157: 1262-1278.
9. Garneau JE, Dupuis ME, Villion M, Romero DA, Barrangou R, et al. (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468: 67.
10. Ishino Y, Krupovic M, Forterre P (2018) History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. J Bacteriol, 200: e00580-17.
11. Gootenberg JS, Abudayyeh OO, Lee JW, Essletzbichler P, Dy AJ, et al. (2017) Nucleic acid detection with CRISPR-Cas13a/C2c2. Science 356: 438-442.
12. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna, JA, et al. (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816-821.
13. Horvath P, Barrangou R (2010) CRISPR/Cas, the immune system of bacteria and archaea. Science 327: 167-170.
14. Doudna JA, Charpenteir E (2014) The new frontier of genome engineering with CRISPR-Cas9. Science 346: 1258096.
15. Cong L, Ran FA, Cox D, Lin S, Barretto R, et al. (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339: 819-823.
16. Prashant M, Yang L, Kevin ME, John A, Marc G, et al. (2013) RNA-guided human genome engineering *via* Cas9. Science 339: 823-826.
17. Ye L, Wang C, Hong L, Sun N, Chen D, et al. (2018) Programmable DNA repair with CRISPRa/i enhanced homology-directed repair efficiency with a single Cas9. Cell Discovery 4: 46.
18. Jiang W, Marraffini LA (2015) CRISPR-Cas: New tools for genetic manipulations from bacterial immunity systems. Annu Rev Microbiol 69: 209-228.
19. Komor AC, Badran AH, Liu DR (2017) CRISPR-based technologies for the manipulation of eukaryotic genomes. Cell 168: 20-36.
20. Thurtle-Schmidt DM, Lo TW (2018) Molecular biology at the cutting edge: A review on CRISPR/CAS9 gene editing for undergraduates. Biochem Mol Biol Educ 46: 195-205.
21. Choudhary E, Thakur P, Pareek M, Agarwal N (2015) Gene silencing by CRISPR interference in mycobacteria. Nat Commun 6: 6267.
22. Mandegar MA, Huebsch N, Frolov EB, Shin E, Truong A, et al. (2016) CRISPR interference efficiently induces specific and reversible gene silencing in human iPSCs. Cell Stem Cell 18: 541-553.
23. Wensing L, Sharma J, Uthayakumar D, Proteau Y, Chavez A, et al. (2019) A CRISPR interference platform for efficient genetic repression in *Candida albicans*. mSphere 4: e00002-19.
24. Myrbraten IS, Will K, Salehian Z, Havarstein LS, Straume D, et al. (2019)

CRISPR interference for rapid knockdown of essential cell cycle genes in *Lactobacillus plantarum*. mSphere, 4: e00007-19.

25. Sander JD, Joung JK (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32: 347.
26. Albitar A, Rohani B, Will B, Yan A, Gallicano GI (2018) The application of CRISPR/Cas technology to efficiently model complex cancer genomes in stem cells. J Cell Biochem 119: 134-140.
27. Pattanayak V, Lin S, Guilinger, JP, Ma E, Doudna JA, et al. (2013) High-throughput profiling of off-target DNA cleavage reveals RNA programmed Cas9 nuclease specificity. Nat Biotechnol 31: 839-843.
28. Cho SW, Kim S, Kim Y (2014) Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Genome Res 24: 132-141
29. Wu Y, Zhou H, Fan X, Zhang Y, Zhang M, et al (2015) Correction of a genetic disease by CRISPRCas9-mediated gene editing in mouse spermatogonial stem cells. Cell Res 25: 67-79
30. Andrei C, Florin T, Andreea G, Georgiana N, Alina B (2017) Development, applications, benefits, challenges and limitations of the new genome engineering technique. An update study. Acta Medica Marisiensis 63: 4-9
31. Kaulich M, Dowdy SF (2015) Combining CRISPR/Cas9 and rAAV templates for efficient gene editing. Nucleic Acid Ther 25: 287-296.