E-ISSN: 2706-8927
P-ISSN: 2706-8919
www.allstudyjournal.com
IJAAS 2020; 2(4): 336-339
Received: 09-08-2020
Accepted: 15-09-2020

Ezatullah Marefat
Assistant Professor,
Department of Biology,
Faculty of Natural Science,
Shaheed Rabbani Education University, Kabul,
Afghanistan

Corresponding Author:
Ezatullah Marefat
Assistant Professor,
Department of Biology,
Faculty of Natural Science,
Shaheed Rabbani Education University, Kabul,
Afghanistan

The role of CRISPR/Cas9 technology in genomics edit

Ezatullah Marefat

Abstract
Many genoms editing tools have been used successfully to revise and control the genomes of various animals, but endonucleases under the guidance of RNA are known by the title of CRISPR / CAS9; and are naturally changing the genomic engineering domain. Since the introduction of CRISP / Cas9 is much simpler than ZFN or TALEN, which is originated from the simple construction of CRISPR / Cas9 vectors that target a specific place in the host genome. This technology has been changed importantly, quickly and only within a few years as a standard tool for targeted editing the Gene. In this article, I will discuss about that how this technology is formed and how it will be developed in the coming years. Editing genemic strategies by CRISPR / Cas9 will accelerate functional genomic studies in the coming years. CRISPR / Cas9 will not only change genetic engineering, but also various fields of research in biological studies.

Keywords: Genome, edit, endonuclease, restriction enzyme, CRISPR/Cas9

Introductions
Genome's edit with the engineering endonuclease by using of ZFNs and TALENs is theoretically similar to DNA manipulation by restricted endonuclease in laboratories, which causes cuts in DNA. By the means of comparison, the function of these two-component endonucleases is similar to the paintings of the Emologeics in which the initial antibodies conjugate directly to alkaline phosphatase. As we know, two components, means primary antibodies without any conjugation and secondary antibodies that detect primary alkaline phosphatase-binding antibodies that can facilitate immunochemical manipulation; because the secondary antibodies are conjugated with phosphatase and can be used for any primary herantibody and to identify antigen types (Hsu PD *et al.*, 2014) [6, 11, 19]. A similar phenomenon is expected in editing technology. Situational Prokaryotic immunity is similar to a diamond whose beauty is visible after enduring hardships which is shown until we should know about the importance of two component systems for the purposeful Genemic editing.

Cas9 in immunity acquired bacterial
For the first time in archaebacteria, CRSPIR / Cas9 were recognized as a microbial immune system by which this system acquired these acquisitive immunity creatures against viruses and plasmids. According to Gasiunas *et al.* (2012) [5] places of gene CRISPR are observed in 40% of sequenced bacteria and 90% of sequenced Arcs. And there can be more than one place in it for bacteria. When an invasive foreign DNA enters a bacterium, it is broken down by Cas nuclease enzymes, and then part of it is placed in the crisper and between duplicate sequences, in this case it is called a spacer. Spacer sequences are used as templates to generate short RNA (crRNA) sequences and form the complex with the trans-activating CrRNA (tracrRNA). Together, these two sequences direct the Cas9 protein to the aggressive DNA; specifically, binding of the Cas9 protein to the aggressive DNA.
Based on the Hale *et al.* (2009) [5] ideas since the discovery of this system, 45 different Cas protein families have been described in articles and reports, each with different roles in crRNA synthesis, uptake and attachment of new spacer sequences, and aggressive DNA cleavage. The CRISPR / Cas9 system is generally divided into three categories based on the Cas protein sequences and the structure of the genes that make them up.
Type 1, type 2 and type 3. The CRISPR / Cas9 system that has so far been widely used for editing genome is a type 2 Crisper system derived from the bacterium Streptococcus pyogenes (Barrangou *et al.*, 2007) [1].

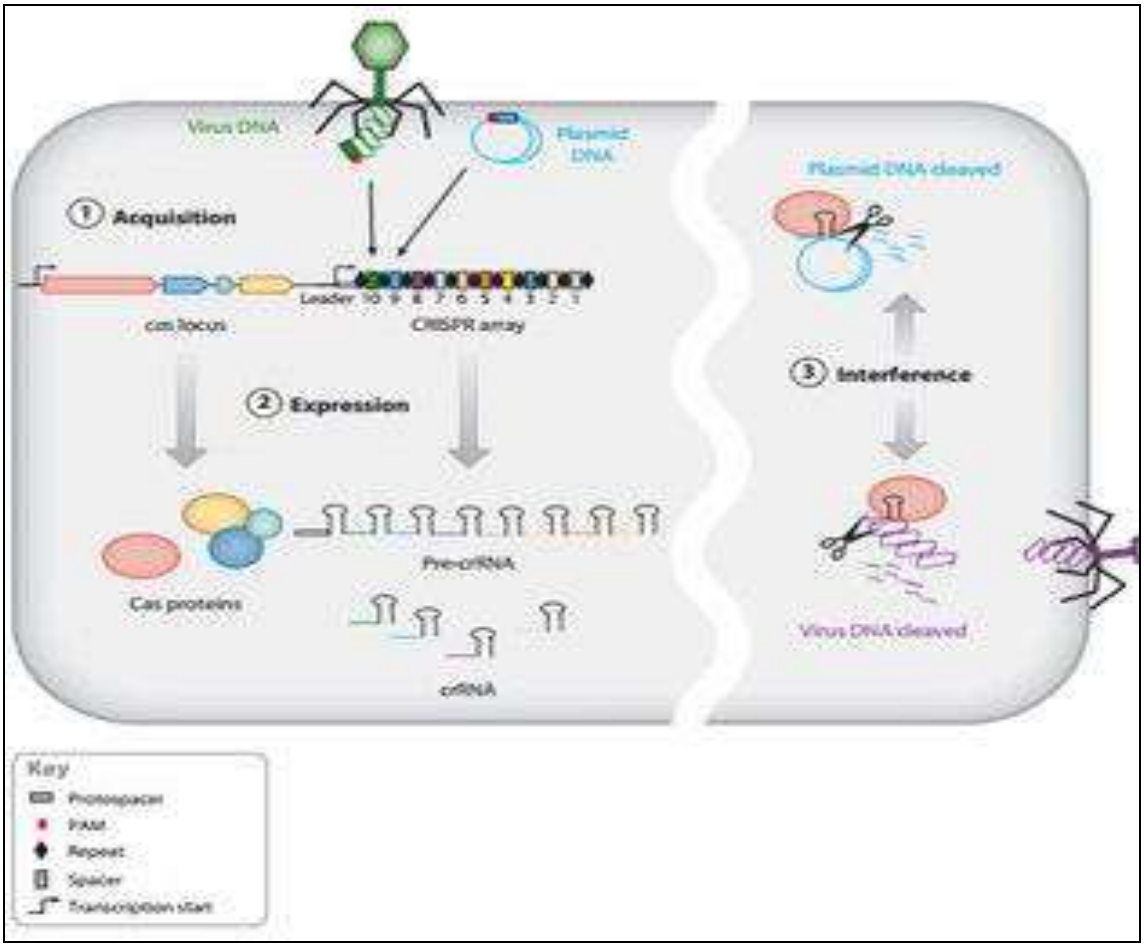

Fig 1: Natural Mechanism of Acquired Immune System CRISPR / Cas9 Type 2. External nucleotides such as DNA or viral plasmids have entered the CRISPR locus of the host genome and show degradation by Cas9 nuclease activity.

Application of CRISPR / Cas9 in Adit or Edit Genoms

When using the CRISPR / CAS9 system in genoma's edit or Adit, only two criteria are required: a guide chimeric RNA (gRNA) that acts similarly to the CrRNA-tracrRNA complex in bacteria and a Cas9 protein with nuclease activity.

Although, the specificity of targeting depends mainly on the gRNA sequence, Cas9 also requires specific Nitrogen base multiples known as a motif adjacent to the main spacer (PAM) (14). PAM sequences vary between types of bacteria. For example, Streptococcus pyogenes (SpCas9) requires (5`-NGG03) (Jinek *et al.,* 2012) [8]. (StCas9) Streptococcus thermophiles Cas9 requires (5`-NNAGAAW-3` (Cong L and Ran FA *et al.,* 2013) [4].

Furthermore, Walsh *et al.* (2013) [17] also added that (NmCas9) Neisseria meningitides is also requires (5`-NNNNGATT-3`). Hsu PD and Lander ES (2014) [7] to support Walsh *et al*. ideas described that SpCas9 is currently widely used for genetic engineering. It is known that at the beginning of the SpCas9-gRNA complex in the genome, it searches for the PAM sequence and then opens the double-stranded DNA strand in a directed manner, resulting in the formation of DNA-RNA pairs (Stremberg *et al.,* 2014) [16]. To cause a double-strand break, the two second nucleases, HNH and RuvC, create a separate split in the Watson-Creek strands, causing a linear double-strand break in nitrogen bases 3 to 4 high pair upstream sequence becoming PAM (Nishmasu *et al.,* 2014) [11].

Therefore, Hsu PD *et al.* (2013) [7] claimed that GRNA structure is another important factor for CRISPR / CAS9-based edit Genome. Although crRNA and tracerRNA can be transcribed separately like the classical CRISPR / Cas system, but, the structure of chimeric gRNA is relatively simple and often leads to high activity. In addition, Sander JD and Joung JK (2014) [14] stated that CRISPR A chimeric gRNA consists of a crRNA-derived region at the 5 'end and a tracrRNA-derived region at the 3` end, and different variations in both regions have been accepted by several groups. Basically, the DNA detection sequence in the crRNA region is 20 pairs of nitrogenous base twin pairs. However, it is said that the addition or subtraction of nitrogenous multiples may improve specificity (Cho *et al.,* 2014) [3]. Also, Kim and Kim (2014) [3, 9] explained that the end of region 3` crRNA and the ends of region 5´ tracrRNA are usually joined together by four nucleotides 5´-GAA-3´ to form a large ring stem known as a tetralope. The tracrRNA region has additional small loops in the 3rd direction, and these sequences are important for high gRNA expression. In addition, in some studies, A-U displacements have been adopted in Poly-A and poly-T regions (Chen *et al.,* 2013) [2].

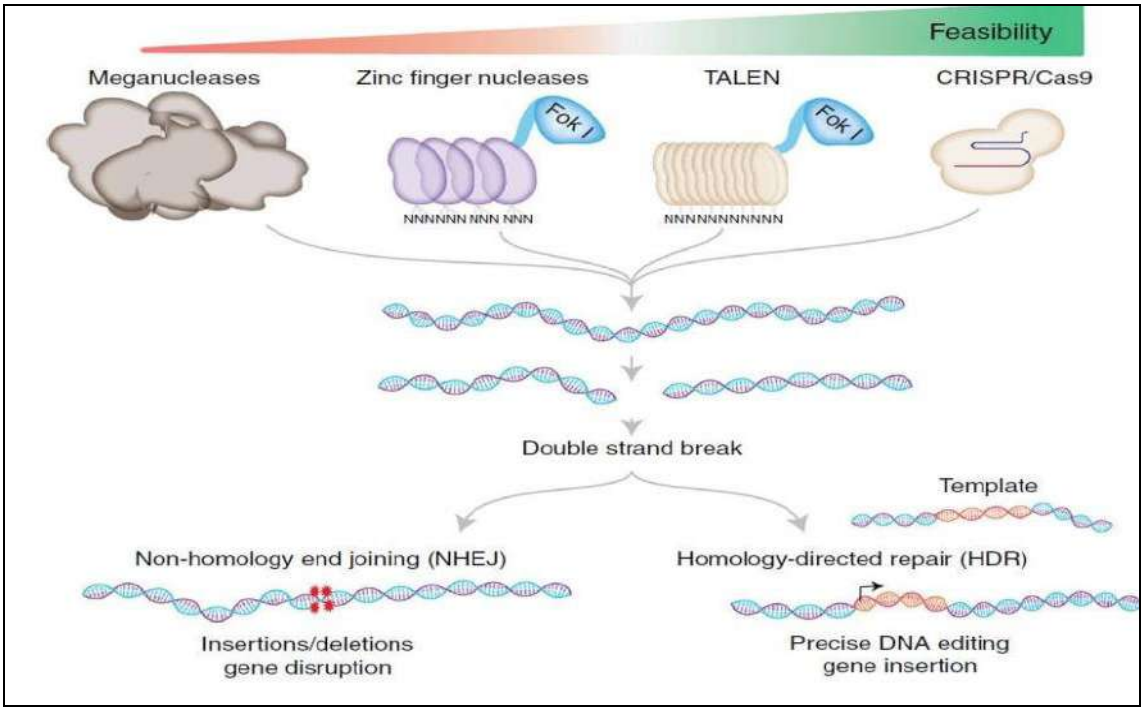

Fig 2: shows the editing of the genome by this system

CRISPR/Cas9 targeting specificity

As described above, approximately 20 pairs of Nitrogen base gRNAs and three sequences of three pairs of PAM games from SpCas9 specify the CRISPR / Cas9 targeting specificity. Due to the PAM sequence, SpCas9 has the ability to bind to 5′-NGA-3′ (Zhang *et al.*, 2014) [6, 18, 20] and 5′-NAG-3′ (Jiang *et al.*, 2013) [7] as well as 5′-NGG-3′. Due to the gRNA targeting sequence, the degree of specificity decreases with increasing distance from the PAM region. A sequence that extends to 12 pairs of died twins on the side of the PAM region is called a seed sequence and has high target specificity in comparison (Nishmasu *et al.*, 2014) [11]. In some types of cells cultured, especially cells such as HEK293T, U2OS and k562, many unwanted recurrent mutations have been observed by many groups. In vitro experiments have also shown unwanted connections with high replication (Pattanayak *et al.*, 2013) [13]. However, in normal cells such as the embryonic stem cells of mice and creatures such as mice and rats, the rate of induced unwanted mutations does not appear to be so high (Wang *et al.*, 2013) [18]. In addition, the complete sequencing of genomes has recently been performed by several groups to analyze unwanted mutations in ginoma cells. The results showed that among the human stem cells treated with CRISPR / Cas9 as well as TALENs, the cell clones had unwanted isolates with low replication (Smith *et al.*, 2014) [15]. These reports suggest that the frequency of mutations in the non-target sequence varies between cell types due to potential differences in the bi-directional failure repair device.

Interestingly, a genomic study of SpCas9-gRNS-binding regions using Immunological chromatin immunosuppression followed by sequencing (ChIP-seq) showed that only seven nucleotides, including 5′-GG-3′ in PAM, could be used to be agreed an identified as sequences (Wu X *et al*, 2013). In addition, although thousands of non-target binding regions were identified, only one potential non-target region produced significant mutations in mouse embryonic stem cells (Zhang *et al.*, 2014) [6, 18, 20]. On the other hand, 70% of SpCas9-gRNA-binding regions were associated with genes (Wu X *et al.*, 2013). This result suggests that changes in transcriptional settings for unwanted gins may have been initiated by CRISPR / Cas9, as various studies have shown that inactive catalytic binding of Cas9 to a coding region or regulatory region inhibits transcriptional level (CRIAPRi) (Zhao *et al.*, 2014) [21]. Although more studies are needed to clarify this, we need to identify the potential for side effects without any mutations when using CRISPR / Cas9.

Targeted adjustment at the transcription level

Most of the attention of molecular researchers in this topic is on the ability to induce failure of two disciplines in the genome and use it for functional studies and therapeutic goals. Therefore, the Cas9 Namira Cells protein can be designed to retain the specificity of binding to the DNA sequence in the target genome, even regardless of nuclease activity. For this reason, the use of chimeric finger proteins on Zinc or TALEs as DNA-binding parts and other catalytic domains that regulate transcription are essential transcriptional control requirements at the genome level. These domains absorbed d with the catalytically inactive Cas9 protein to form CRISPR / Cas9-based transcriptional regulatory regulators (Piatek *et al.*, 2015) [12]

Chimeric proteins actually artificially control transcription at the expression level of one gene and the expression of several genes. As a result, it is able to provide a suitable and powerful substrate for the functional studies of gens and even genomes in their natural environment or under special physiological conditions and developmental stages. Dual controls on gene expression by activators and inhibitors transcribe and alter chromatin. Creating this substrate is a valuable tool for achieving robust functional studies among different types of eukaryotes. In this way, it can be revealed how inactivating a single gene or a set of genes is harmful to the target organism. In addition, today the modifications made by the CRISPR / Cas9 system are widely used in the genome, and extensive studies with minimal nonspecific

targeting show the power of functional studies among eukaryotic species (Konermann *et al.*, 2015) [10].

Conclusion

Today, almost no scientist in the field of molecular biology is covered in biology-related disciplines, and CRISSPR / Cas9 technology is a powerful tool for editing genomes of various organisms. The publication of more than thousands articles since 2010 in this field indicates the importance of this strategic technology in the world. This technology has revolutionized the field of medicine in the field of treatment of genetic diseases through gene therapy. Establishing more than a dozen major companies in the United States and Europe since 2014 with a focus on human genetic therapy using CRISPR / Cas9 technology is promising new horizons in the treatment of deadly genetic diseases such as muscular dystrophy or viral infections such as HIV and hepatitis.

References

1. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, *et al.* CRISPR provides acquired resistance against viruses in prokaryotes. Science 2007;315:1709-12.
2. Chen B, Gilbert LA, Cimini BA, Schnitzbauer J, Zhang W, Li G-W, *et al.* Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas System. Cell 2013;155(7):1479-91.
3. Cho SW, Kim S, Kim Y, Know J, Kim HS, Bae S, *et al.* Analysis of off-target effects of CRISPR/Cas- derived RNA-guided endonucleases and nickases. Genome research 2014;24(1):132-41.
4. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, *et al.* Multiplex genome engineering using CRISPR/Cas systems. Science 2013, 1231143.
5. Gasiunas G, Barrangou R, Horvath P, Siksnys V. (2012). *Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc. Natl Acad. Sci.* USA **109**, E2579–E2586.
6. Hale CR, Zhao P, Olson S, Duff MO, Graveley BR, Wells L, *et al.* RNA-guided RNA cleavage by a CRISPR RNA-Cas protein complex. Cell 2009;139:945-56.
7. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell 2014;157(6):1262-78.
8. Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA. RNA–guided editing of bacterial genomes using CRISPR/ Cas systems. Nature biotechnology 2013;31:(3):233.
9. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual- RNA-guid DNA endonuclease in adaptive bacterial immunity. Science 2012, 1225829.
10. Kim H, Kim JS. A guide to genome engineering with programmable nuclease. Nature Reviews Genetic 2014;15(5):321- 34.
11. Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, *et al.* Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature 2015;517:583-8
12. Nishmasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N, *et al.* Crystal structure of Cas9 on in complex with guided RNA and target DNA. Cell 2014;156(5)935-49.
13. Piatek A, Ali Z, Baazim H, Li L, Abulfaraj A, Al- Shareef S, *et al.* RNA- guided transcriptional regulation in plantavia synthetic dCas9- based transcription factors. Plant Biotechnol 2015;13:578-89.
14. Pattanayak V, Lin S, Guilinger JP, Ma E, Doudna JA, Liu DR. High - throughput profiling of off- target DNA cleavage reveals RNA-programmed Cas9 nuclease specificity. Nature biotechnology 2013;31(9):839.
15. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. Nature biotechnology 2014;32(4):347-55.
16. Smith C, Gore A. Yan W, Abalde- Atristain L, Li, He C. *et al.* Whole genome sequencing analysis reveals high specificity of CRISPR/Cas9 and TALEN- based genome editing in human iPSCs. Cell stem cell 2014;5:4240.
17. Stremberg SH, Reading S, Jinek M, Greene EC, Doundna JA. DNA interrogation. By the CRISPR RNA-guided endonuclease Cas9. Nature 2014;507(7490):62.
18. Walsh RM, Hochedlinger K. A variant CRISPR/Cas9 systems adds versatility to genome engineering. Proceeding of The national academic of Sciences: 2013;110(9):156443(9).
19. Wang H, Yang H, Shivalila CS, Dawlaty MM. Change AW, Zhang F. *et al.* One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediate genome engineering. Cell 2013;153(4):910-8.
20. Wu X, Scott DA. Kriz AJ, Chiu AC, Hsu PD, Dadon DB, *et al.* Genome–wide binding of the CRISPR endonuclease Cas9 in mammalian cells. Nature biotechnology 2014;32(7):670.
21. Zhang Y, Ge X, Yang F, Zhang L, Zheng J, Tan X, *et al.* Comparision of non- canonical PAMs for CRISPR/ Cas9- mediated DNA cleavage in human cells, Scientific reports 2014;4:5405.
22. Zhao Y, Dai Z, Liang Y, Yin M, Ma K, He M, *et al.* Sequencing – Specific inhibition of microRNA via CRISPR/ CRISPRi systems. Scientific report 2014;4:3943.