BioCore
Exploring Scientific Community

International Journal of Biotechnology and Bioengineering

ISSN 2475-3432

Research Article Open Access

CRISPR/Cas-9 System: Magnificent Tool for Genome Editing

KavitaVasdev*, Indra Mani

Department of Microbiology, Gargi College, University of Delhi, Siri Fort Road, New Delhi, India

Corresponding author: KavitaVasdev, Department of Microbiology, Gargi College, University of Delhi, Siri Fort Road, New Delhi -110049, India. Tel:+91-11-26494544, E-mail: atvikav@gmail.com

Citation: KavitaVasdev et al. (2017), CRISPR/Cas-9 System Magnificent Tool for Genome Editing. Int J Biotech & Bioeng. 3:9, 302-306. DOI: 10.25141/2475-3432-2017-9.0293

Received: May 13, 2017; **Accepted:** May 24, 2017; **Published:** November 24, 2017

Abstract

The recent development of clustered regularly interspaced short palindromic repeats (CRISPR)is a key technology for genome editing, targeting, and regulation in a wide range of organisms and cell types. The CRISPR features may be exploited for typing purposes, epidemiological studies, host-virus ecological surveys, building specific immunity against undesirable genetic elements, and enhancing viral resistance in domesticated microbes. Microbes rely on various defense mechanisms, which allow them to resist viral predation and exposure to invading nucleic acid. In several bacteria and most archaea, CRISPR form peculiar genetic loci, which provide acquired immunity against viruses and plasmids by targeting nucleic acid in a sequence-specific manner. CRISPR-Cas9 is an RNA-mediated adaptive immune system that protects bacteria and archaea from viruses or plasmids. In this review, we emphasize the current progress and the future potential of the CRISPR-Cas9 system towards biomedical, therapeutic, industrial, and biotechnological applications.

Keywords: CRISPR-Cas9, Genome Editing, CRISPRi, RNAi

Introduction:

The progress of effective and consistent ways to make particular, targeted changes to the genome of living cells has been a long-standing aim of biomedical researchers (Cong et al. 2013). Recently, a new tool based on a bacterial CRISPR-associated protein-9 nuclease (Cas9) from *Streptococcus pyogenes* has generated extensive attention. This follows several attempts over the years to manipulate gene function, including homologous recombination (Capecchi, 2005) and RNA interference (RNAi) (Fire, et. al., 1998).RNAi, in specific, became a laboratory principle enabling inexpensive and high-throughput interrogation of gene function (Martinez, et. al., 2003), however it is disadvantaged by providing only temporary inhibition of gene function and unpredictable off-target effects (Alic, et.al., 2012).

The functions of CRISPR and CRISPR-associated (Cas) genes are vital in adaptive immunity in leading bacteria and archaea, enabling the organisms to respond to and eliminate invading genetic material (Figure 1).These repeats were primarily noticed in the 1980s in *E. coli* (Ishino, et. al., 1987) and later Barrangou et. al.,(2007), demonstrated that *S. thermophilus* can acquire resistance against a bacteriophage by integrating a genome fragment of an infectious virus into its CRISPR locus.

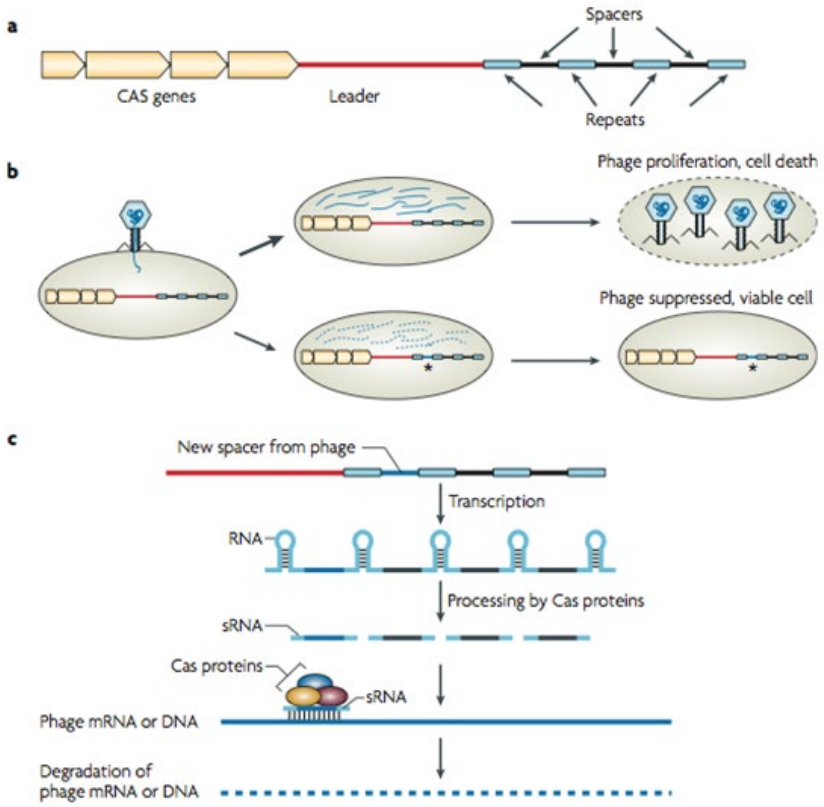

Figure 1: CRISPR structure and function.

a) Typical structure of a clustered regularly interspaced short palindromic repeat (CRISPR) locus.

b) CRISPRs acquire phage-derived spacers that provide immunity. Following an attack by a phage, phage nucleic acids proliferate in the cell and new particles are produced, leading to the death of the majority of the sensitive bacteria. A small number of bacteria acquire phage-derived spacers (marked by an asterisk), leading to survival, presumably by CRISPR- mediated degradation of phage mRNA or DNA.

c) Putative simplified model for CRISPR action. The repeat-spacer array is transcribed into a long RNA, and the repeats assume a secondary structure. Cas proteins recognize the sequence or structure of the repeats and process the RNA to produce small RNAs (sRNAs), each of which contains a spacer and two half repeats. The sRNAs, complexed with additional Cas proteins, base pair with phage nucleic acids, leading to their degradation. Putatively, this process is mediated by one or more of the Cas proteins. CAS, CRISPR-associated (Soreket. al., 2008).

The CRISPR/Cas system of immunity is comprised of three steps; adaptation, expression, and interference. The adaption stage involves the recognition and cleavage of a protospacer from invading DNA by the cas genes. The subsequent insertion (acquisition) of foreign DNA (spacers) into the CRISPR locus is also referred to as spacer acquisition or immunization. The expression stage refers to the expression of relevant cas genes and their proteins leading to the transcription of the CRISPR array into a long RNA molecule called the precursor CRISPR RNA (pre-crRNA). Cas proteins and other accessory factors then process this further into short mature crRNA. In the final interference stage, this mature crRNA and other cas proteins recognize foreign nucleic acid and destroy it. This is also referred to as the immunity stage, which these mechanisms mimic (Makarovaet. al., 2006).

Three types of CRISPR mechanisms have been identified, of which type II is the greatest studied. In this case, invading DNA from viruses or plasmids is cut into small fragments and incorporated into a CRISPR locus during a series of short repeats. The loci are transcribed, and transcripts are then processed to produce small RNAs (crRNA–CRISPR RNA), which are used to monitor effector endonucleases that target invading DNA based on sequence complementarity(Jinek, et. al., 2012).

Targeting effectiveness, or the ratio of desired mutation accomplished, is one of the ultimate significant parameters by which to evaluate a genome-editing technique. The targeting effectiveness of Cas9 compares favorably with more recognized approaches, such as Transcription activator-like effector nucleases (TALENs), or Zinc-finger nucleases (ZFNs)(Mussolino, et. al., 2011). For instance, in human cells, custom-designed ZFNs and TALENs could only accomplish productivities ranging from 1% to 50% (Mussolino, et. al., 2011). However, the Cas9 technology has been described to have effectiveness up to >70% in zebrafish(Hwang, et. al., 2013) and plants (Feng, et. al., 2013), and ranging from 2–5% in induced pluripotent stem cells (Mali, et. al., 2013). Moreover, Zhou, et. al.,(2014) improved genome targeting up to 78% in one-cell mouse embryos, and accomplished effective germline transmission through the use of dual sgRNAs to concurrently target asingle gene.

The pinnacle of four decades of research, induced pluripotent stem cells (iPSCs), and genome editing with the advent of CRISPR now

promise to take drug development and regenerative medicine to new levels and to enable the interrogation of disease mechanisms with a previously unimaginable level of model fidelity (Waddington, et. al., 2016).

Current trends in CRIPR/Cas-9

Succeeding its first demonstration in 2012 (Ishino, et. al., 1987), the CRISPR/Cas9 technology has been extensively implemented. Furthermore, CRISPR/Cas9 technology has also been successfully expended to target key genes in numerous cell lines as well as organisms, including **HIV-1**(Ebina, et. al., 2013), **HBV**(Kennedy,et al., 2015), **HPV18**(Kennedy et al., 2014), **HPV16**(Kennedy,et al., 2014), **HCV** (Price,et al., 2015), bacteria (Fabre, et. al., 2014), yeast (DiCarlo, et. al., 2013), C. elegans (Hai, et. al., 2014), Drosophila (Gratz, et. al., 2014), zebrafish(Hwang, et. al., 2013), *Xenopus tropicalis* (Guo, et. al., 2014), monkeys (Niu, et. al., 2014), rabbits (Yang,et. al., 2014), pigs (Hai, et. al., 2014), rats (Ma, et. al., 2014), mice (Mashiko, et. al., 2014), human (Mali, et. al., 2013) and plants (Mali, et. al., 2013).

Numerous research groups have been taken advantage of this method to introduce single point mutations (deletions or insertions) in a particular target gene, via a single gRNA (Miller, et. al., 2011). A recent past and current great development is the use of the dCas9 version of the CRISPR/Cas9 technology to target protein domains for microscopic visualization of specific genome loci (Chen, et. al., 2013), transcriptional regulation (Perez-Pinera, et. al.,2013) and epigenetic modification (Hu, et. al., 2014).

The CRISPR/Cas9 technology requires only the redesign of the crRNA to modify target specificity. This differentiates it from the other additional genome editing tools, comprising ZFNs and TALENs, where redesign of the protein-DNA interface is essential. Besides, CRISPR/Cas9 permits prompt genome-wide interrogation of gene function by generating large gRNA libraries (Koike-Yusa, et. al., 2013) for genomic screening.

The future prospects of CRISPR/Cas9

The rapid progress in developing CRISPR/Cas9 into a set of tools for cell and molecular biology research has been notable, likely due to the simplicity, high efficiency and versatility of the system. Recombination-based tools for introducing targeted genomic mutations in *Mycobacterium tuberculosis* are not efficient due to higher rate of illegitimate recombination compared with homologous DNA exchange. Also, involvement of multiple steps and specialized reagents make these tools cost ineffective. However, CRISPRinterference (CRISPRi) demonstrated that this approach efficiently represses expression of target genes in Mycobacteria. It has shown that co-expression of the codon-optimized dCas9 of S. pyogenes with sequence-specific guide RNA results in complete repression of individual or multiple targets in Mycobacteria. Therefore CRISPRi offers a simple, rapid and cost-effective tool for selective control of gene expression in mycobacteria(Chaudharyet. al., 2015).Moreover, simplicity of use, low cost, high speed, multiplexing potential, and equal or higher specific DNA targeting ability have secured its popularity and success across the global scientific community (Mali,et.al., 2013;Strauβ et. al., 2013;Rahdaret. al., 2015).

The key advantages of the CRISPR-Cas system are its ability to genetically modify an organism without leaving any foreign DNA behind and its versatility and simplicity of programming. Contrasting the reprogramming of its predecessors, ZFNs and TALENs, which require editing of DNA-interacting domains located at different sites on the DNA-binding scaffolds, CRISPR-Cas systems changes are only executed on the recombinant RNA sequences (Travis 2015; Rahdaret. al., 2015).Furthermore this practical aspect, the major drawback the CRISPR-Cas system faces is restrictive legislation. The power of this gene-editing tool has caused concerns to wider society, due to the potential for irrevocable alteration of future generations, if used in germ line alterations.

In specific, the first report on the use of CRISPR-Cas9 on human tripronuclear zygotes was published in 2015. These zygotes have one oocyte nucleus and two sperm nuclei and are therefore unable to develop into viable embryos. It was this work that encouraged a worldwide moratorium by both biologists and ethicists on human germ line genetic engineering (Hurlbut 2015). Though this study showed low homologous recombination events of the human β-globin (HBB) gene, for which the CRISPR-Cas9 system was designed, as well as mosaicism and off-target cleavage at various sites, it was the first time CRISPR-Cas9 effectively cleaved endogenous genes on human embryos (Liang,et. al., 2015).

Fear that one of CRISPR-Cas‘major advantages, its power for “democratization of gene targeting” (Hotta 2015) could be unsafe if used by the wrong people to enhance population minorities and reignite an interest for eugenics, maybe addressed by strong international jurisdictions and global public engagement (Liang et. al., 2015;Chan et. al., 2015; Krishanet. al., 2015). However, before this could be performed, a deeper understanding of appropriate models (both cellular and animal, as well as human embryonic stem/iPS cell-derived germ line cells) to test efficacy and safety and multigenerational effects, and optimization of genome editing tools to minimize off-target events will be necessary. These include the development of more accurate and sensitive tools to assess off-target events and mosaicism (Chan et. al., 2015).

In conclusion,the potential of the CRISPR-Cas system could be explored for the treatment and cure of critical human diseases i.e., cancer, diabetes, cardiovascular etc. Due to its high potential in therapeutics by altering the genome *in vivo*, CRISPR-Cas technology’s application directly on humans may raise certain ethical issues, which can be dealt with by formulation of proper legislation, after due discussions and debates, balancing the benefit of technology for improving human life to avoiding its misuse. The promise that this technology holds, calls for continued promotion and support for improvements through research to allow maximum advantage of this magical tool for the benefit of humankind .

References

1. Alic, N, Hoddinott, M.P., Foley, A., Slack, C., Piper, M.D.W. and Partridge, L.(2012). Detrimental Effects of RNAi: A Cautionary Note on Its Use in Drosophila Ageing Studies. PLoSOne, 7,e45367.

2. Barrangou, R., Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero, D. A., Horvath P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. Science, 315,

1709–1712.

3. Capecchi, M.R. (2005). Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. Nat. Rev. Genet. 6, 507–512.

4. Chan S, Donovan PJ, Douglas T, et al. (2015). Genome editing technologies and human germline genetic modification: the Hinxton group consensus statement. Am J Bioeth. 15(12): 42–7.

5. Chaudhary, E., Thakur, P., Pareek, M and Agarwal, N. (2015). Gene silencing by CRISPR interference in mycobacterium. Nature Communications. 6:6267.

6. Chen, B., Gilbert L. A., Cimini BA, Schnitzbauer J, Zhang W, Li, G.W., Park J, Blackburn, E. H., Weissman, J. S., Qi, L. S., Huang B.(2013).Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system.Cell, 155, 1479–1491.

7. Cong L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffini, L. A., Zhang, F.(2013). Multiplex genome engineering using CRISPR/Cas systems. Science, 339:819–823.

8. DiCarlo, J.E., Norville, J.E., Mali, P., Rios, X., Aach, J., Church, G.M. (2013). Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems. Nucleic Acids Res. 41, 4336–4343.

9. Ebina, H., Misawa, N., Kanemura, Y., Koyanagi, Y., (2013). Harnessing the CRISPR/Cas system to disrupt latent HIV-1 provirus. Sci. Rep. 3, 2510.

10. Fabre, L., Hello, S.L., Roux, C., Issenhuth-Jeanjean, S. and Weill, F. X. (2014). CRISPR Is an Optimal Target for the Design of Specific PCR Assays for Salmonella enterica Serotypes Typhi and Paratyphi A. PLoSNegl.Trop.Dis.8: e2671.

11. Feng, Z., Zhang, B., Ding, W., Liu, X., Yang, D.L., Wei, P., Cao, F., Zhu, S., Zhang, F., Mao, Y., Zhu, J.K. (2013). Efficient genome editing in plants using a CRISPR/Cas system. Cell Res. 23:1229–1232.

12. Fire, A., Xu, S., Montgomery, MK., Kostas, SA., Driver, S. E.& Mello, C.C.(1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature, 391, 806–811.

13. Gratz, S.J., Ukken, F.P., Rubinstein, C.D., Thiede, G., Donohue, L.K., Cummings, A.M., O'Connor-Giles, K.M. (2014). Highly specific and efficient CRISPR/Cas9-catalyzed homology-directed repair in Drosophila. Genetics. 196(4): 961-71.

14. Guo, X., Zhang, T., Hu, Z., Zhang, Y., Shi, Z., Wang, Q., Cui, Y., Wang, F., Zhao, H., Chen, Y. (2014). Efficient RNA/Cas9-mediated genome editing in Xenopustropicalis. Development, 141, 707–714.

15. Hai, T., Teng, F., Guo, R., Li, W., Zhou, Q. (2014). One-step generation of knockout pigs by zygote injection of CRISPR/Cas system. Cell Res. 24(3): 372-5.

16. Hotta, A., Yamanaka, S. (2015). From genomics to gene therapy: induced pluripotent stem cells meet genome editing. Annu Rev Genet. 49:47–70.

17. Hu, J., Lei, Y., Wong, W.K., Liu, S., Lee, K.C., He, X., et al. (2014). Direct activation of human and mouse Oct4 genes using engineered TALE and Cas9 transcription factors. Nucleic Acids Res. 42(7): 4375-90.

18. Hurlbut, J.B. (2015). Limits of responsibility: genome editing, asilomar, and the politics of deliberation. Hastings Cent Rep. 45(5): 11–4.

19. Hwang, W.Y., Fu, Y., Reyon, D., Maeder, M.L., Kaini, P., Sander, J.D., Joung, J.K., Peterson, R.T., Yeh, J.R. (2013). Heritable and precise zebrafish genome editing using a CRISPR-Cas system. PLoS One, 8:e68708.

20. Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., Nakata, A.(1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J. Bacteriol. 169, 5429–5433.

21. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., Charpentier, E.(2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science, 337:816–821.

22. Kennedy, E.M., Bassit, L.C., Mueller, H., Kornepati, A.V., Bogerd, H.P., et al., (2015). Suppression of hepatitis B virus DNA accumulation in chronically infected cells using a bacterial CRISPR/Cas RNA-guided DNA endonuclease. Virology, 476, 196–205.

23. Kennedy, E.M., Kornepati, A.V., Goldstein, M., Bogerd, H.P., Poling, B.C., et al., (2014). Inactivation of the human papillomavirus E6 or E7 gene in cervical carcinoma cells by using a bacterial CRISPR/Cas RNA-guided endonuclease. J. Virol. 88 (20), 11965–11972.

24. Koike-Yusa, H., Li Y, Tan EP, Velasco-Herrera Mdel C, Yusa K. (2013). Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library. Nat. Biotechnol. 32(3): 267-73

25. Krishan, K., Kanchan, T., Singh, B. (2015).Human genome editing and ethical considerations. SciEng Ethics.

26. Liang, P., Xu, Y., Zhang, X., et. al.,(2015). CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell. 6(5):363–72.

27. Ma, Y., Zhang, X., Shen, B., Lu, Y., Chen, W., Ma, J., Bai, L., Huang, X., Zhang, L.(2014). Generating rats with conditional alleles using CRISPR/Cas9.Cell Res. 24: 122–125.

28. Makarova, K.S., Grishin, N.V., Shabalina, S.A., Wolf, Y.I., Koonin, E.V. (2006). A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. Biol Direct. 1:7.

29. Mali, P., Yang, L., Esvelt, K.M., Aach, J., Guell, M., DiCarlo, J.E., Norville, J.E., Church, G.M.(2013). RNA-guided human genome engineering via Cas9. Science, 339:823–826.

30. Martinez, J., Patkaniowska, A., Elbashir, S.M., Harborth, J., Hossbach, M., Urlaub, H., Meyer J, Weber K, Vandenburgh K, Manninga H, Scaringe SA, LuehrmannR, Tuschl T, (2003). Analysis of mammalian gene function using small interfering RNAs. Nucleic Acid. Res. Suppl. 3:333.

31. Mashiko, D., Young SA, Muto M, Kato H, Nozawa K, Ogawa M, Noda T, Kim YJ, Satouh Y, Fujihara Y, Ikawa M. (2014). Feasibility for a large scale mouse mutagenesis by injecting CRISPR/Cas plasmid into zygotes. Dev. Growth Differ. 56, 122–129.

32. Miller, J.C., Tan, S, Qiao, G., Barlow, K.A, Wang, J. et. al. (2011). A TALE nuclease architecture for efficient genome editing. Nat. Biotechnol. 29:143–148.

33. Mussolino, C., Morbitzer, R., Lütge, F., Dannemann, N., Lahaye, T., Cathomen, T. (2011). A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity. Nucleic Acids Res. 39:9283–9293.

34. Niu, Y., Shen, B., Cui, Y., Chen, Y., Wang, J., Wang, L.et. al. (2014). Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell. 156:836–843.

35. Perez-Pinera, P., Ousterout, D.G., Brunger, J.M., Farin, A.M., Glass, K.A., Guilak, F., Crawford, G.E., Hartemink, A.J., Gersbach, CA. (2013). Synergistic and tunable human gene activation by combinations of synthetic transcription factors. Nat. Methods, 10:239–242.

36. Price, A.A., Sampson, T.R., Ratner, H.K., Grakoui, A., Weiss, D.S. (2015). Cas9-mediated targeting of viral RNA in eukaryotic cells. Proc. Natl. Acad. Sci. U. S. A. 112 (19), 6164–6169.

37. Rahdar, M., McMahon, M.A., Prakash, T.P., Swayze EE, Bennett CF, Cleveland DW. (2015). Synthetic CRISPR RNA-Cas9-guided genome editing in human cells. ProcNatlAcadSci U S A. 112(51):E7110–7.

38. Sorek, R., Kunin, V and Hugenholtz P (2008).CRISPR — a widespread system that provides acquired resistance against phages in bacteria and archaea. Nature reviews. 6:181-186.

39. Strauβ, A. and Lahaye, T. (2013). Zinc fingers, TAL effectors, or Cas9-based DNA binding proteins: what's best for targeting desired genome loci? Mol Plant. 6(5): 1384–7.

40. Travis, J. (2015). Making the cut. Science. 350(6267): 1456–7.

41. Waddington, S. N., Privolizzi, R., Karda, R. and O'Neill, HC (2016).A Broad Overview and Review of CRISPR-Cas Technology and Stem Cells. Curr Stem Cell Rep (2016) 2:9–20

42. Yang, D., Xu J, Zhu T, Fan J, Lai L, Zhang J, Chen YE.(2014). Effective gene targeting in rabbits using RNA-guided Cas9 nucleases. J. Mol. Cell Biol. 6:97-99.

43. Zhou, J., Wang J, Shen B, Chen L, Su Y, Yang J, Zhang W, Tian X, Huang X. (2014). Dual sgRNAs facilitate CRISPR/Cas9-mediated mouse genome targeting. FEBSJ. 281(7): 1717-25.