THE SCIENCE WORLD
A Monthly e Magazine
ISSN:2583-2212
July, 2023; 3(07), 1274-1278

Popular Article

CRISPR-Cas9 based genome editing with particular emphasis on Veterinary Parasitology

[1]Bhupamani Das*, [2]Abhinav Suthar and [3]H.R. Parsani
[1,2]Assistant Professor and [3]Associate Professor & Head
[1,3]Department of Veterinary Parasitology, [2]Department of Veterinary Medicine
College of Veterinary Science & A.H., Kamdhenu University, Sardarkrushinagar, Gujarat-385506
https://doi.org/10.5281/zenodo.8133828

Abstract

In the field of parasitology, CRISPR/Cas9 technology is advancing quickly and enabling molecular processes to be dissected with unmatched efficiency. Structural and functional genomics information helps in investigation of the relationship between parasite and their host. CRISPR based genome editing tool have found to be successful on several parasite including *Leishmania donovani, Toxoplasma gondii, Trypanosoma cruzi, Strongyloides stercoralis, Cryptosporidium* spp.

Keywords: CRISPR, Genome editing, DNA, Parasitology

Introduction

Parasites pose a serious threat to human and animal health. In fact, parasite disease has hampered the growth of livestock and, consequently, economic development in many parts of the world. *Theileria parva, Schistosoma mansoni, Trypanosoma cruzi, T. brucei, and Leishmania spp.* are just a few of the parasites whose genomes have been better sequenced and understood as a result of these developments. The study of functional genomes is becoming more and more important in the field of parasitology. Additionally, the analysis of a parasite's relationship to its host using structural and functional genomics will be made possible by genome sequence information. New diagnostic techniques for parasite infections and the creation of vaccines and antiparasitic drugs may result from a better understanding of these relationships (Cui and Yu, 2016).

Genome editing or gene editing or genome engineering is a type of genetic engineering in which DNA is modified in the genome of a living organism. The recently developed cutting-edge genome editing technique known as CRISPR enables researchers to work within cells and make exact changes to

any DNA sequence (Zheng *et al.,* 2014). CRISPR denotes clustered regularly interspaced short palindromic repeats where Cas9 (CRISPR associated protein 9) is an enzyme that uses CRISPR sequences as a guide to recognize and cleave specific DNA complementary to the CRISPR sequences. In Tokyo, Japan, Jennifer Doudna and Emmanuel Charpentier received the Japan Prize in 2017 for their ground-breaking development of CRISPR-Cas9. CRISPR is astonishingly easier, quicker, and less expensive than any other gene editing methods that have been previously discovered.

Classification of CRISPR-Cas systems: The variety of CRISPR-Cas systems has quickly increased since their discovery. Currently, CRISPR-Cas systems may be classified into two classes, six kinds, and a number of subtypes 20 based on evolutionary relationships. Class 1 systems are characterized by a complex of many effector proteins, whereas class 2 systems include a single crRNA-binding protein. Class 2 systems, which are the easiest to reconstruct of the many CRISPR systems, have mostly been used for diagnostic purposes. Class 1 systems have also been developed for diagnostics, either in conjunction with elements of the class 2 system or with the native type III complex.

How CRISPR –Cas9 system works? CRISPR was first discovered in *Escherichia coli* and was not created by scientists; rather, it was inspired by bacteria (Ishino *et al.,* 1987). The three phases of the CRISPR/Cas system's processes are adaptation, expression, and interference. At the 5′-terminus of the CRISPR locus, protospacers from the foreign DNA are integrated during the first phase. Precursor non-coding RNA, or pre-crRNA, is produced by CRISPR during the second step of the process. After that, the short-chain crRNA is produced from the long-chain pre-crRNA. At the type II system, Cas9 is crucial at this phase. In order to direct Cas-mediated cleavage, the short-chain crRNA and tracrRNA combine to produce a double-stranded secondary structure. Binary complexes of the RNAs cr and trac interact to the Cas protein in the third phase, which is the final step. Depending on the sequence of the short guide RNA (sgRNA), which is normally obtained from the crRNA/tracrRNA, the Cas protein can be re-programmed to cut different targets. These complexes recognize certain loci and cut the double-stranded foreign DNA to produce double-stranded breaks. This system is a flexible tool for gene editing because it is very responsive to tailoring that enables it to specifically target nucleic acid sequences (Figure 1.0).

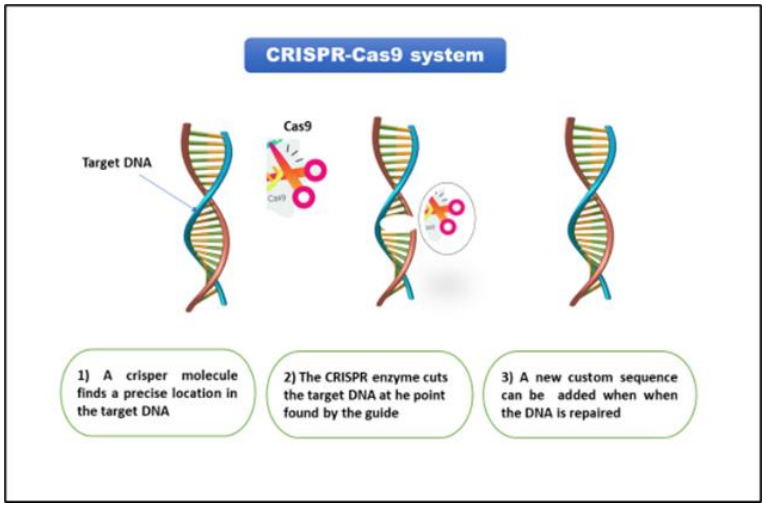

Figure 1.0: Mechanism of CRISPR-Cas9 system
(Photo courtesy *Bhupamani Das)*

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
*Published:*11.07.2023

Application of CRISPR-Cas9 system: To date, CRISPR-Cas9 system has been used in various studies including identification of new drug target, understanding parasite vector interaction, vaccine development and drug resistance (Figure 2.0). The various CRISPR-Cas9 related work over the years in different parasite has been summarized in the Table 2.0.

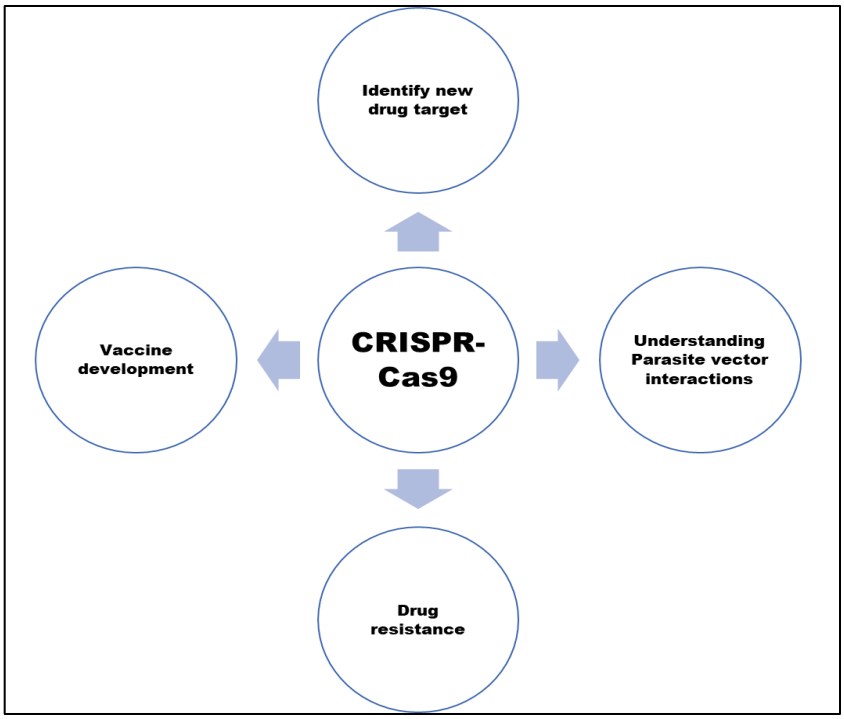

Figure 2.0: Applications of CRISPR-Cas9 system in Veterinary Parasitology

(Photo courtesy *Bhupamani Das)*

Table 1.0: Work done on CRISPR-Cas9 system in some economically important parasites

Disease	Work done	Reference
Leishmaniasis	CRISPR-Cas9-mediated genome editing in *Leishmania* species	Engstler and Beneke, (2023).
Trypanosomosis	Knockout studies using CRISPR-Cas9 to study parasite	Peng *et al.* (2015)
Toxoplasmosis	Knockout studies in *Toxoplasma gondii* using CRISPR/Cas9	Zheng *et al.* (2014)
Cryptosporidiosis	CRISPR/Cas9 based knockout studies in *Cryptosporidium parvum*	Funkhouser-Jones *et al.* (2023)
Strongyloidosis	Transgenesis in *Strogyloides*	Lok *et al.* (2017)
Malaria	Resistance studies in Plasmodium falciparum	Sene *et al.* (2023)
Schistosomosis	CRISPR/Cas9-mediated editing in *Schistosoma mansoni* for functional genomics studies	Du, 2023

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
*Published:*11.07.2023

Advantages of current CRISPR-Cas9 system: Out of the three main genome editing tools, TALENs (Transcription Activator-Like Effector Nucleases), Mega nucleases, and Zinc Finger Nucleases are the most expensive, complex to customize, and synthesize, as well as requiring the most advanced knowledge and experimental setups. Being a straightforward and flexible method, CRISPR/Cas9 has a number of advantages over other genome editing tools. The fact that CRISPR/Cas9 does not require protein engineering, a difficult task when compared to other genome editing technologies, is one of its main advantages. Compared to developing a protein for a particular target site, testing and designing gRNA is easier. Additionally, a large library of gRNA that may be exploited for high-throughput functional genomics applications can be created utilizing just 20 nucleotides of gRNA. CRISPR/Cas9 is also ideally suited for multitasking. Finally, researchers with tight research resources can employ this technology because it is affordable. With all of these benefits, this innovative approach has become a widely used technique in genome editing research labs (Satsya *et al.*, 2018).

Limitations of CRISPR-Cas9 system: Despite being a ground-breaking technology, CRISPR/Cas9 has some intrinsic limitations. The off-target effect' is CRISPR/Cas9's most serious issue. A gRNA can cause mutation at locations that are similar to the target site but not exactly the same. These off targets are challenging to find, and the only method that might work would be a genome-wide search for places that have sequence similarities to the target site. However, attempts are being made to address the issue of being "off target" with regard to the successful deployment of this technology in the near future (Satsya *et al.*, 2018).

Conclusion

Although CRISPR/Cas9 genome editing is well-established in the realm of parasites and insect vectors, efforts are still being made to boost its effectiveness and expand its applications. To make the most of this ground-breaking technology in each of our sectors, prompt communication and ongoing advice- and idea-sharing are essential. In the future, it will be vital to both apply the CRISPR toolset to novel approaches and to debug conventional genome-editing applications. Finally, CRISPR/Cas9 is being utilized to carry out larger-scale functional screenings in economically important parasitic disease. This has the potential to develop knockout/knockdown libraries, a useful resource for the parasitology community.

References

Cui, Y. and Yu, L. (2016). Application of the CRISPR/Cas9 gene editing technique to research on functional genomes of parasites. *Parasitology international*, *65*(6 Pt A), 641–644. https://doi.org/10.1016/j.parint.2016.08.011

Du, X. (2023). Programmed CRISPR/Cas9-mediated editing to generate Schistosoma mansoni mutants as a blueprint for functional genomics studies of schistosomes.

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com
*Published:*11.07.2023

Engstler, M. and Beneke, T. (2023). Gene editing and scalable functional genomic screening in Leishmania species using the CRISPR/Cas9 cytosine base editor toolbox LeishBASEedit. *Elife*, *12*, e85605.

Funkhouser-Jones, L. J., Xu, R., Wilke, G., Fu, Y., Schriefer, L. A., Makimaa, H. and Sibley, L. D. (2023). Microbiota-produced indole metabolites disrupt mitochondrial function and inhibit *Cryptosporidium parvum* growth. *Cell Reports*, *42*(7).

Ishino, Y., Shinagawa, H., Makino, K., Amemura, M. and Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *Journal of Bacteriology*, 1987; 169:5429-5433

Lok, J. B., Shao, H., Massey, HC. and Li, X. (2017). Transgenesis in Strogyloides and related parasitic nematodes: historical perspectives, current functional genomics applications and progress towards gene disruption and editing. *Parasitology*, 144:327-342.

Peng, D., Kurup, S. P., Yao, P. Y., Minning, T. A. & Tarleton, R. L. (2015). CRISPR-Cas9-mediated single- gene and gene family disruption in *Trypanosoma cruzi*. *Molecular Biology*, 6:e02097 e02114.

Sastya, S., Chouhan, M., Karunakaran, V., and Jamra, N. (2018). Role of CRISPR/Cas9 gene editing tool in parasitology: A review. *Journal of Entomology and Zoological Studies*, *6*(4), 243-246.

Sene, S. D., Pouye, M. N., Martins, R. M., Diallo, F., Mangou, K., Bei, A. K. and Mbengue, A. (2023). Identification of an in vitro artemisinin-resistant *Plasmodium falciparum* kelch13 R515K mutant parasite in Senegal. *Frontiers in Parasitology*, *2*, 1076759.

Zheng, J., Jia, H. and Zheng, Y. (2014). Knockout of lucine aminopeptidase in *Toxoplasma gondii* using CRISPR/Cas9. *International Journal of Parasitology*.

Official Website
www.thescienceworld.net
thescienceworldmagazine@gmail.com

***Published:*11.07.2023**