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CRISPR-Cas9 System: The Future of Genome Editing Studies

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ABSTRACT

Recent discovery of CRISPR-Cas9 genome editing technique has opened up a new window for biotechnologists. The simplicity along with its accuracy and cost effectiveness has made the technique instant hit for modern day researchers. But the full potential of the technique is yet to be explored as there are some serious challenges and ethical issues needed to be addressed elaborately. In this article the development of CRISPR-Cas9 system has been described along with its special features , detailed discussion on the future directions and challenges has also been made.

Keywords: Genome editing, CRISPR-Cas9, Nuclease, Human germline editing, Disease modeling, Ethical issues

The term "Genome Editing" can be defined as precise and efficient modification of DNA (de-oxyribonucleic acid) within a cell. Genome editing can be used for altering, removing or adding some part of DNA and subsequently changing some characteristics of a cell or organism. Several molecular tools like Site-directed nucleases (SDNs), Zinc finger nuclease (ZFNs) and Transcription activator-like effector nucleases (TALENs), Oligonucleotide-directed mutagenesis, RNA interference etc. have been used by modern day molecular biologist for effective and precise genome editing but it is the discovery of CRISPR-Cas system which has struck the scientific community like a storm for its simplicity, high fidelity, cost effectiveness as well as ethical issues related to it. The CRISPR-Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated) technology has gained such worldwide popularity in the scientific community that in 2015, Science Magazine recognized "CRISPR genome editing" as breakthrough of the year. The present paper will highlight important technical aspects, application potential of the CRISPR-Cas system along with some social and ethical issues related to the approach.

It has been found that the CRISPR system is an adaptive immune mechanism present in many bacteria and the majority of characterized Archaea. Organisms which contain CRISPR acquire DNA fragments from invading bacteriophages and plasmids, transcribes them into CRISPR RNAs (crRNAs) and guide cleavage of invading RNA or DNA Wang H *et al.* (2016). There are CRISPR adjacent genes which are extremely important for their activity which are named Cas genes (CRISPR-associated). An estimate of 45 Cas gene families have been predicted by a comparative genomics study of bacterial and archaeal genome.

Among them only Cas1 & 2 have been found commonly associated with all 45 Cas families, both of which are involved in spacer acquisition systems Waddington S N *et al.* (2016). Based on differences in their components and mechanisms of action, CRISPR systems have been divided into two major classes

Makarova K.S. *et al.* (2015). RNA guided target cleavage in class 1 systems (types I, III, and IV) requires a large complex of several effector proteins, but in the class 2 systems [type II, putative types Zetsche V.B. *et al.* (2015) and Shmakov V.I. (2015)], only one RNA-guided endonuclease [e.g., Cas9 in type II and Cpf1 (CRISPR from Prevotella and Francisella-1) in type V] is required to mediate cleavage of invading genetic material. In general, a CRISPR system works in three stages: 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 Makarova K S *et al.* (2006). Cas9 is the RNA-guided endonuclease that cleaves target DNA in the class 2 type II CRISPR system, is the most widely used for genomic editing and regulation among the Cas proteins and due to its extensive use the system is commonly referred to CRISPR-Cas9 system.

In a scientific article Waddington S N *et al.* (2016) pointed out that the main 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. Unlike 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 J (2015). Ease 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 P *et al.* (2013).

A Brief History of CRISPR-Cas9 System Discovery

In 1993, Francisco Mojica was the first researcher to characterize what is now called a CRISPR locus and along with Ruud Jansen Mojica coined the term CRISPR.

In 2005, he reported that these sequences matched snippets from the genomes of bacteriophage Mojica *et al.* (2005). Bolotin was first to identify a novel Cas gene whose protein product had nuclease activity, which is now known as Cas9 Bolotin *et al.* (2005). Bolotin was also instrumental in the recognition of the target motif for Cas-9 nuclease which is called the protospacer adjacent motif (PAM) Bolotin *et al.* (2005). It was Koonin who hypothesized the scheme for CRISPR cascades as bacterial immune system based on inserts homologous to phage DNA in the natural spacer array. It was later proved by Horvath and colleagues experimentally that CRISPR systems are indeed an adaptive immune system: they integrate new phage DNA into the CRISPR array, which allows them to fight off the next wave of attacking phage Barrangou *et al.* (2007).

Another piece of critical information came from John van der Oost and colleagues who showed that in *E-scherichia coli*, spacer sequences, which are derived from phage, are transcribed into small RNAs, termed CRISPR RNAs (crRNAs), that guide Cas proteins to the target DNA Brouns *et al.* (2008). Some key understanding about the mechanism of interference came from Marraffini and Sontheimer, who elegantly demonstrated that the target molecule is DNA, not RNA Marraffini and Sontheimer (2008).

Then Moineau and colleagues demonstrated that CRISPR-Cas9 creates double-stranded breaks in target DNA at precise positions, 3 nucleotides upstream of the PAM Garneau *et al.* (2010). Emmanuelle Charpentier confirmed that Cas9 is the only protein required for cleavage in the CRISPR-Cas9 system and in addition to the crRNA, a second small RNA exists, which they called trans-activating CRISPR RNA (tracrRNA) Deltcheva *et al.* (2011). Siksnys and colleagues cloned the entire CRISPR-Cas locus from

S. thermophilus (a Type II system) and expressed it in *E. coli* (which does not contain a Type II system), for precisely elucidated its capability of providing plasmid resistance Sapranauskas *et al.* (2011). Siksnys and colleagues undertook a series of biochemical experiments to mechanistically characterize Cas9's mode of action Gasiunas *et al.* (2012). Zhang, who had previously worked on other genome editing systems such as TALENs, was first to successfully adapt CRISPR-Cas9 for genome editing in eukaryotic cells Cong *et al.* (2013). They also showed that the system could be programmed to target multiple genomic loci, and also could drive homology-directed repair. It was also reported by a group of researchers from George Church's lab at Harvard University in the same issue of Science Mali *et al.* (2013).

Applications in Genome Editing

Several studies have already successfully demonstrated the potential of CRISPR-Cas9 system in genome editing. For highly virulent strains of bacteria Cas9 has been applied immunologically as an antimicrobial agent and has been developed to specifically target antibiotic resistance Mojica F J. *et al.* (2009), Schwank G. *et al.* (2013). Cells from human patients with cystic fibrosis showed functional repair of the CFTR gene in vitro in cultured intestinal stem cell organoids using CRISPR-Cas Schwank G. *et al.* (2013). Tyrosinemia,a hereditary disease which is caused by a defective gene has been corrected with the help of CRISPR technology in mice Yin H. *et al.* (2014). Treatment of viral infections such as HIV and hepatitis B has also been demonstrated using Cas9 Zhen S. *et al.* (2014), Hu W. *et al.* (2014). CRISPR technology was implemented in carrying out genetically modified cynomolgus monkeys Niu Y. *et al.* (2014). In a path breaking research study published in Protein & Cell Liang P. *et al.* (2015) used tripronuclear (3PN) zygotes to further investigate CRISPR/Cas9-mediated gene editing in human cells. This particular study has opened a new door of controversy in the global scientific community on whether human germ line editing studies are necessary for genetic enhancement or not.

CRISPR-Cas9 system has also been used in editing genomes of crop plants. In a recent study Wang *et al.* (2014b) used both TALEN and CRISPR/Cas9 technologies to target the genes of the mildew-resistance locus (MLO) in wheat and successfully knocked out all three MLO homoeoalleles, generating plants resistant to powdery mildew disease. Ainley W.M. *et al.* (2013) in their paper proposed that site-specific nucleases also allow targeted molecular trait stacking, i.e., the addition of several genes in close vicinity to an existing transgenic locus. Once stacking has been achieved, the entire array of transgenes can be mobilized into other germplasm by crossing because it behaves as a single locus. Another application of CRISPR-Cas9 that is likely to expand in the future is the targeted insertion of transgenes in the fields of metabolic engineering and molecular farming, where plants or plant cells are used as factories for the production of specific metabolites or proteins Bortesi L. *et al.* (2015).

Apart from its role in genome editing CRISPR-Cas9 system could be broadly used in reverse genetics studies to understand the role of specific genes, for disease modeling, and for demonstrating new therapeutic schemes in a number of models of genetic and infectious diseases as suggested by Xiao-Jie L. *et al.* (2015), Hsu P.D. *et al.* (2014) and Doudna J.A. (2014).

Final Outlook

CRISPR-Cas9 system is a very powerful genome editing tool that has been discovered by modern biologists. The technology has yet to reach its full potential and it is sure that in near future scientists will explore the huge amount of genomic and systems biology data with this technique. From basic science researchers to clinicians the CRISPR-Cas9 technology is going to serve extensively. But there are concerns also; recent exploits from a Chinese group of scientists using CRISPR-Cas9 to edit the human embryo genome was not completely successful. So, it had to be abandoned at its preliminary stage Liang P. *et al.* (2015). Various scientific groups have expressed their concern about human germ line

editing studies which raises serious ethical issues and challenges. Scientists now demand a temporary moratorium should be imposed on the technology and a common platform should also be developed among researchers and stakeholders to discuss the future roadmap for successful use of the technology.

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