CRISPR-Cas9: Components and Application

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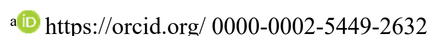
Abstract: The identification of components of the CRISPR system discovered in bacterial cells enables gene editing in a more efficient way. The further upgrading allows researchers to easily edit the DNA sequence not only in prokaryotic cells but also in mammalian cells. The novelization of the CRISPR-Cas9 system promotes broader utilization of this technique, resulting in staggered cuts in dsDNA sequences. Even during the COVID-19 pandemic, the employment of CRISPR-Cas9 techniques accelerates the development of SARS-CoV-2 testing kits, allowing fast and effective testing available to a massive population. In this paper, I describe the progress of finding the components followed by the novelization as well as applications of the CRISPR-Cas9 system.

1 INTRODUCTION

An impassioned discussion about the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) system has been initiated because of the Nobel Prize winner Emmanuelle Charpentier and Jennifer Doudna, the pioneers of CRISPR technology, in 2020 (Savić, Schwank 2016). Early in 1987 when CRISPR has been discovered for the first time, a sequence located in the bacterial genome that disarms bacteriophages infection by cutting off the phage's DNA (Doudna, Charpentier 2014). This bacterial adaptive immune system was first being used for genome editing in 2012 when Charpentier and Doudna thoroughly discover the feature of the CRISPR-Cas9 system which can recognize a specific sequence of DNA and cut off the target site (Savić, Schwank 2016). The nuclease Cas 9 followed by two guide RNA sequences together constitute the genetic scissor (Savić, Schwank 2016). Since then, they were also able to target different DNA sequences by reprograming the sequence of the short RNA (Doudna, Charpentier 2014). Scientists are now able to genetically modify the sequence of short RNA and insert that RNA inside Cas 9 protein to target and disable the desired sequence of DNA in cells (Zhang et al. 2014). Engineering not only the sequence of the guide RNA but also the protein Cas 9 enable scientists to add, delete, disable, enhance, or even replace the sequence of interest much more easily than other genome editing techniques such as Zinc Finger Nuclease (ZFN), and Transcription-Activator Like Effector Nucleases (TALEN) (Gupta et al. 2019).

This precious research achievement brings not only people in the science field, but ordinary people's attention towards the application, advantages, challenges, and potential shortcomings of this technique. Thousands of questions have been delivered to scientists to answer but some of them have not yet been responded systematically.

In this review, with the aim of collectively and comprehensively discussing why and how the CRISPR-Cas 9 system deserves this massive attention, the content of introduction of the CRISPR-Cas 9 system, potential applications of CRISPR technology, the progress of cutting-edge technology, and the future opportunities of CRISPR-Cas9 will be included. The significance of the objective focuses on providing a thorough introduction of the CRISPR-Cas9 system as well as its up-to-date application to promote learning, thinking, and potential breakthrough within this field.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2 CRISPR-Cas9 SYSTEM

2.1 The Understanding of CRISPR-Cas9

CRISPR-Cas9 was first understood by Jennifer Doudna and Emmanuelle Charpentier in 2012 (Jinek et al. 2012). Before 2012, Dr. Doudna worked on understanding and analyzing the composition of the CRISPR-Cas9 system which has been found out to be involved in the bacterial innate immune system to defend against viral infection (Wiedenheft et al. 2009). Working along with Dr. Charpentier, Dr. Doudna identified the inner component of Cas9 protein consisted in the type II CRISPR-Cas9 system (Hryhorowicz et al. 2017).

Cas9 is a DNA endonuclease guided by two RNAs, one is called CRISPR RNAs (crRNAs) and the other is trans-activating crRNAs (tracrRNA) (Jinek et al. 2012, Cong et al. 2013). crRNA is complementary to the sequence that Cas9 is targeting, while tracrRNA is essential to crRNA maturation as well as triggering plasmid cleavage (Jinek et al. 2012). A specific motif appears in the foreign genome called protospacer adjacent motif (PAM), 2-6 nucleotides downstream of the cut site, also need to be recognized by crRNA, and cut by Cas9 3-4 nucleotide upstream of it (Jiang, Doudna 2017). Once part of the sequence is fully recognized, Cas9 protein bind onto the strand, making the cut using two domains, each domain cut one DNA strand (Jinek et al. 2012) (Fig. 1a). By utilizing radioactivating tags on one of the DNA strands, Doudna et al. determined that the HNH domain is responsible for complementary strand cleavage, while the RuvC-like domain is for the uncomplimentary strand cleavage (Jinek et al. 2012). A linear dsDNA is obtained after the cleavage showing a successful cut by Cas9 (Jinek et al. 2012).

Although this is a part of the innate immune system of multiple bacteria such as *Streptococcus pyogenes*, the research found out that the CRISPR-Cas9 system is programmable and can be used in other organisms by changing the guide RNA (Chen et al. 2019). Moreover, crRNA and tracrRNA can be linked together with a hairpin to become a chimeric RNA that mimics crRNA: tracrRNA complex (Chen et al. 2019, Ran et al. 2013). Studies show that by editing the sequence of chimeric RNA, Cas9 protein with a single chimeric guide RNA is potentially capable of cutting any dsDNA in many organisms, resulting in a new era of genomic regulation and genomic editing (Chen et al. 2019, Ran et al. 2013).

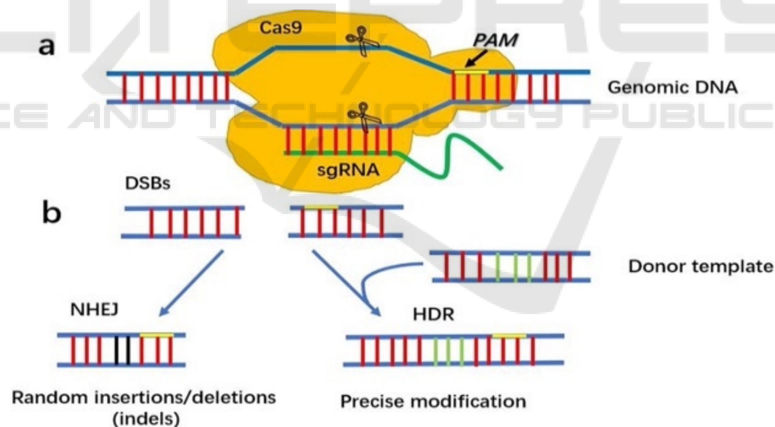

Figure 1. The CRISPR-Cas9 system. a. Double strand DNA breaks and binds to Cas9 and single guided RNA. Each domain cut one strand of the DNA and resulting in double-strand break of genetic material (Chen et al. 2019). The original circular DNA will become linear DNA due to the cut (Jinek et al. 2012). b. The double-strand lesions will be repaired through either a non-homologous end joining or a homologous repair pathway. Both NHEJ and HDR allow the desired sequence to be eliminated from the DNA achieving the goal of attacking foreign DNA (Chen et al. 2019, Ran et al. 2013, Cong et al. 2013).

2.2 Repurposing CRISPR-Cas9 System

The CRISPR-Cas9 system is programmed not only to cut dsDNA but also to manipulate transcription in eukaryotes (Qi et al. 2013). Inactive Cas9 or denatured Cas9 (dCas9) protein is a great model in the experiment (Cong et al. 2013) (Fig. 2). In this case, the Cas9 lacking the endonucleolytic activity can normally bind to complementary strand DNA but cannot cut desired sequence [Qi et al. 2013]. Research has shown that while Cas9 binds to the dsDNA, the transcription is blocked with low off-target effects (Qi et al. 2013, Hsu et al. 2014). Furthermore, Cas9 has

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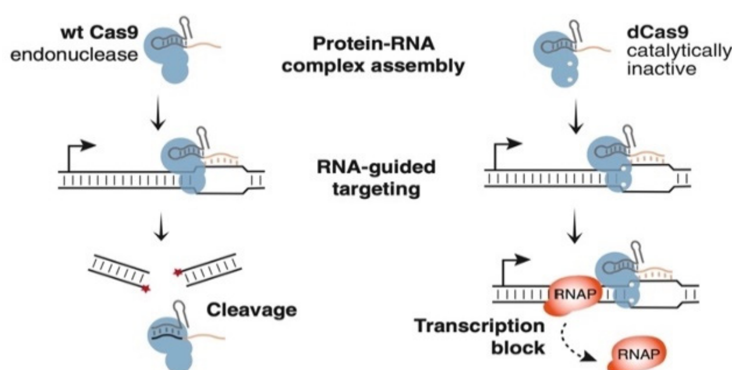

Figure 2. The programmable Cas9 protein and single-guide RNA (sgRNA) enable transcription regulation (Qi et al. 2013). The wild type Cas9 protein cut results in the forming of linear DNA (left), while a dead Cas9 protein with a modified sgRNA can bind onto the DNA sequence and shut down the original transcription by occupying the transcriptional site where the RNA polymerase (RNAP) needs to bind (right) (Qi et al. 2013).

been used in multiple eukaryotic organisms to test the efficiency and accuracy of genetic regulation and as a result, the outcome is positive in most of the organisms (Hsu et al. 2014).

The system originated from bacteria is used to cut off and silence viral DNA (Jinek et al. 2012, Ran et al. 2013). Silencing of the desired segment of dsDNA in eukaryotic cells is achieved by cutting off the target sequence followed by adding more nucleotide base pairs to close the gap (Ran et al. 2013). Cas9 cutting resulting in target double-strand DNA breaks induces non-homologous end-joining or homologous repair pathway (Chen et al. 2019, Cong et al. 2013, Hsu et al. 2014) (Fig. 1b). Both pathways contribute to precise gene editing by silencing a specific gene transcription with very low off-target effects. Furthermore, research carried out in mammalian cells indicated that the CRISPR-Cas9 system could also show high efficiency of creating target DNA lesions with the desired sgRNA (Hsu et al. 2014).

Cong et al. contributed significantly to the understanding and further investigation of the type II CRISPR-Cas9 system in human cells (Cong et al. 2013). Human codon-optimized *Streptococcus pyogenes* Cas9 (SpCas9) and RNase III (hSpRNase III) are modified versions of Cas9 and RNase that can be better used in human cells and at the same time have less error with higher efficiency (Cong et al. 2013).

According to Cong et al., the type II CRISPR-Cas9 system can be as efficient and accurate as of the TALENs which has been used in genetic editing prior to the discovery of CRISPR technology (Cong et al. 2013). Furthermore, they claimed that CRISPR-Cas9 system can do multiplexed genome engineering (Cong et al. 2013). Cas9 is engineered to target both EMX1- and PVALB loci. The efficiency is 1.6%, indicating the ability to do multiplexed editing in a single genome (Cong et al. 2013). The efficiency of cutting is diminished by mismatches between the sgRNA and target DNA (Slaymaker et al. 2015). Neutralization of positively charged groove between HNH, RuvC, and PAM interacting domains on the nontargeting DNA strand promotes rehybridization between dsDNA strands and hence encourages tight binding between sgRNA and target DNA strand (Slaymaker et al. 2015). This upgrade by Skaymaker et al. allows CRISPR-Cas9 system to reach a newer level with a very low off-target effect in terms of target cutting (Cong et al. 2013, Slaymaker et al. 2015).

2.3 Novelizations and Progression of the Application of CRISPR-Cas9 System

More modifications on the Cas9 system by Zetsche et al. enabled more various binding and cutting. For example, a novel version of Cas9 called Cpf1 and C2c2 endonuclease achieved a staggered cut on dsDNA (Zetsche et al. 2015).

Another significant update on CRISPR-Cas system by Gootenberg et al. was using the method of specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) to detect foreign strains in small amounts such as the infection by Ziska and Dengue virus (Gootenberg et al. 2017). The advantage of this technique is that it can sense viral and bacterial pathogens in samples that are relatively advisable such as human serum or urine samples (Gootenberg et al. 2017). Due to the high accuracy of the technique, it can distinguish between similar viral or bacterial strains successfully (Gootenberg et al.

2017). It can also distinguish SNP in human genotyping (Gootenberg et al. 2017).

During the COVID-19 pandemic, Joung et al. discovered STOPCovid, a technique used to rapidly detect the genetic material of SARS-CoV-2 from oral or nasal swab samples giving either positive or negative outcomes using detection strips or specific values of viral RNA counts under fluorescence readers (Joung et al. 2020). The overall sensitivity is 93.1% and specificity is 98.3% among 402 candidates (Joung et al. 2020).

The rapid, inexpensive, and sensitive detection enables CRISPR-Cas system to be highly used since 2015 in genetic regulation and genomic editing (Gootenberg et al. 2017, Joung et al. 2020).

Recently, studies have shown that the success of CRISPR-Cas9 technology inspired therapy in Huntington's disease, sickle cell diseases, cancer, and B-thalassemia, indicating more potential treatments available in the future targeting different diseases (György 2021).

3 DISCUSSION

Owing to the concern of creating genetic mutation due to permanent change in genes in human cells by the CRISPR-Cas9 technology, it is better having safely regulate the on-and off-switches of the CRISPR-Cas9 system (Shivram et al. 2021). In case of observing relatively high off-target effect that may lead to serious genetic defects as well as unpredictable diseases of an organism, having types of "emergency shut down system" is necessary for more complicated gene editing events, especially when introducing DNA cut and gene segment replacement in mammalian cells or even in human cells. Regulators from *Escherichia coli* and *Salmonella typhi* are identified as having H-NS and LRP which are responsible for negative regulation of Cas expression, while LeuO can positively regulate Cas promoter to further simulate CRISPR-Cas cutting (Shivram et al. 2021). Those genes, in theory, are responsible for regulating Cas system, as well as being potential switches for CRISPR-Cas9 system, but the future investigation is needed to make sure those factors found in bacteria can be equally beneficial with high efficiency in mammalian cells. Moreover, even though the off-target effect is significantly lowered by Zhang et al. after several steps of upgrading, it is not possible for now to achive 0 off-target effects and as a result, it is still unknown if the efficiency can promote a positive outcome in clinical trials (Cong et al. 2013, Zetsche et al. 2015).

4 CONCLUSION

The existence of components located in CRISPR-Cas9 system allow further investigation and advancement in other fields. The revelation of the ability to practice precise cut by RuvC-like and HNH domain, engineer sgRNA, and replicate the mechanism in eucaryotic cells results in a higher frequency of the usage of the CRISPR-Cas9 technique in labs regarding the aim of doing gene editing. This technique is known as the most rapid and the easiest method in genetic regulation and genomic engineering. Not only the Noble Prize winner Emmanuelle Charpentier and Jennifer Doudna but also other scientists progressively and largely contribute to the refinement of the CRISPR-Cas9 system in their research fields. Based on all of the existing gene editing products by the CRISPR-Cas9 system, investigating an efficient way of fully controlling the on and off of CRISPR-Cas9 is critial as well as worth studying in order to make CRISPR-Cas9 a precise gene editing machinery. This paper about CRISPR-Cas9, an influential topic in the science field, hopefully, will promote thinking as well as more discoveries that may lead to better and novel disease treatments in the future.

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