CRISPR-Cas9: A detail on specific tool for gene editing or targeting

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Abstracts:
Objective - The applicability in reverse genetics studies, animal model experiments and also in curing diseases caused by viruses like HIV makes CRISPR-Cas9 system a highly desirable technique with the ability to achieve quick plausible outcomes. The bacterial cells chop up the viral genome. The bacterial cells chop up the viral genome. And it search of these pieces of spacer DNA. The spacer DNA is nothing but pieces of different viral genome. That had infected the cell previously. So each time of bacteriophage infect the cell take it pieces and search of spacer DNA. The interference is between the specific Cas protein and RNA Sequence have going to be merge together. The Crispr system would be somehow like adopted immune system in human. it would kind of memory to prevent this same bacteriophage for infecting the cell other time.
Purpose (Hypothesis) – this article main purpose to describe the Genome editing enabled by the development of tools for make precise, targeted changes to the genome of living cells.
Method (Technique) - CRISPR-Cas9.
Conclusion-The CRISPR-Cas9 is the key of genetic revaluation era. This tool are very usefull to studying the genetic code. at finally in this review both in modeling and the CRISPR/Cas9 system have emerged as a feasible tool to achieve what has seemed impossible for decades.
Summary:

1. **Introduction**
2. **Mechanism**
3. **Application**
4. **Benefits**
5. **Conclusion**

Keywords: CRISPR- Cas9, Bacteria's, Viruses, DNA and RNA.

Abbreviations: CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat, PAM: Protospacer Adjacent Motif, NHEJ: Non-Homologous End Joining, DNA: Deoxyribonucleic acid, RNA: Ribonucleic acid, TALEN: transcription-activator likes effector nucleases, ZFN: zinc-finger nucleases.

Introduction:

What is CRISPR-Cas9?

Clustered Regularly Interspaced Short Palindromic Repeat or CRISPR are a clustered family of short repeats of DNA that forms an integral component of the prokaryotic (bacteria and archaea)

adaptive immune system. The study on CRISPR/Cas9 system began with the findings of repetitive segments of DNA in E. coli in the year 1987 but their function was confirmed later on by Barrangou and coworkers in 2007.

CRISPR System: The discovery of the CRISPR loci began in 1987 when Ishino et al. [1] This System is firstly found in prokaryotic or bacterial cell. The Crispr system is like a type of immune system in prokaryotic or bacterial cell. It has to be found in the bacteria and archaea to adaptive immune system in vertebrates, providing a genetic library (memory) of phages and plasmids that previously have invaded the bacteria. This resistance against foreign nucleic acids after the subsequent infection is assured due to the enzymatic activity of the Cas proteins [2]. Various approaches have been taken to enhance HDR in order to improve the rate of high-fidelity genome edits. Both of the above-mentioned DNA repair pathways compete with each other and, therefore, the inhibition of NHEJ can increase the rate of HDR Maruyama et.al. [3].

- **Crispr locus:** Crispr locus is the repeats of DNA. This repeats first upon short and secondary of palindromic. The palindromic mean that if read it left to right and right to left in the end of the same sequences. And there repeats it regularly interspace it mean that they're spacer DNA between them and this spacer DNA's are regular.

- **DNA repeats** with **spacer DNA** between: so this we called Crispr locus. Crispr repeats are between 27 and 37 bp and spacer DNA is between 32-38 bp.

- **CRISPR System work:** The bacteriophage is virus and this virus can infect the bacterial cell. It attaches the bacterial call then injects the genome in bacterial cell. Now this viral genome to produce viral protein and viral enzyme and then it well change whole change machinery in the bacterial cell. Now the bacterial cell because crispr system can prevent happen in second time.so the Crispr system is somehow like adopted immune system in human. So it kind of memory to prevent this same bacteriophage for infecting the cell other time.

Mechanism:
Immune system: The CRISPR System is three step mechanism.

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[3] Maruyama, T.; Dougan, S.K.; Truttmann, M.C.; Bilate, A.M.; Ingram, J.R.; Ploegh, H.L. Increasing the efficiency of precise genome editing with CRISPR-Cas9 by inhibition of nonhomologous end joining. Nat. Biotechnol. 2015, 33, 538–542. [CrossRef] [PubMed]

1. Adaptation: Cas1 alone can integrate only a small number of spacers and Cas2 alone cannot integrate any. High performance acquisition therefore requires Cas1 and Cas2 together [4].Non-CRISPR proteins such as RecBCD in E. coli and Csn2 in S. thermophiles may also be recruited for adaptation [5]. The protein complex consists of two Cas1 subunits on either end of a Cas2 dimer or two regions in the center, called the 'arginine clamp' and the 'arginine channel', used to stabilize the protospacer. It has a curved binding surface that stretches the length of the spacer to be integrated, acting as a molecular ruler to preserve uniformity of the CRISPR locus sequence architecture. Non-specific sequence binding resulted from the phosphodiester interactions between the protospacer and Cas proteins [6].The primary sequence of the first DNA repeat is crucial for having the CRISPR array nicked to incorporate a new spacer [7]

A. Spacer Acquisition:
The Spacer Acquisition in the same three different types of Crispr system. The bacteriophage infected cell for first time. The bacterial cells chop up the viral genome. And take pieces of this and it search of these pieces of spacer DNA. The spacer DNA is nothing but pieces of different viral genome. That has infected the cell previously. So each time of bacteriophage infect the cell take it pieces and search of spacer DNA. In near of Crispr locus Cas genes (Cas-enzymes) are many enzymes and many enzymes are implicated in the Crispr process. The Cas enzymes in general are nucleuses or helicases.it cut the link of nucleotide. Well helicases can cut the H- bond between the two strains can separate the DNA from each other. Most of the Cas-enzymes are nucleases and helicases in the spacer acquisition we have two main players – Cas1 and Cas2.
Both of them are dimers that can form complex together.

Cas1: It can be has nuclease and integrase activity so it can cut the viral genome and integrate the piece of genome the spacer DNA.

Cas2: its Endoribonucleases Cas2 mainely cut to RNAs.

B. CrRNA Processing: CrRNA is Crispr RNA processing it have Crispr locus different pieces of bacteriophages of spacer DNA. One of the two strains of DNA to transcribe in to mRNA. Now this mRNA is exactly complementary to lower strand so it content complementary sequences.
So there are three types:

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[7] Arslan Z, HermannsV, Wurm R, WagnerR and Pul Ü 2014 Detection and characterization of spacer integration intermediates in type IE CRISPR-Cas system Nucl. Acids Res. 42 7884–93

Type 1: Crispr loop and then the mRNA will be cut choosing cas6e or cas6f enzyme. Mrna this sequences going to be chopped up like this. These small pieces are the CrRNA.
Type 2: To we have another player in this Crispr processing is called tracrRNA (Trans activating crispr RNA).There this pieces of RNA which are bound to the Crispr Sequences mRNA and then the mRNA is chopped up by Cas9 and RNase3.
Type 3: It cas6 Homolog is going to chop up them mRNA in directly end up the CrRNAs. Containing the Crispr Repeats and viral genome.

2. Expression:
After acquisition, spacers are transcribed as crRNAs to guide effector modules for invader interference. Long precursor crRNA (pre-crRNA) transcripts are processed from the CRISPR array and cleaved into the individual crRNAs by Cas enzymes in most systems and by an endogenous endoribonuclease in Type II systems. Interestingly, a streamlined functional architecture for crRNA maturation was discovered in the Neisseria meningitidis Type II-C locus [[8]].a CRISPR DNA repeat binding protein (Cbp1) involved in regulating the production of pre-crRNA transcripts also exists [[9]].The tracrRNA was discovered from RNA sequencing of Streptococcus pyogenes and had a 24-nucleotide complementarity to pre-crRNA repeat regions [[10]].The tracrRNA binds to Cas9 (formally Csn1) to facilitate base-pairing with the pre-crRNA's repeats and promotes pre-crRNA cleavage into crRNA by an endogenous endoribonuclease III (RNase III) [[11]]

3. Interference: In general CrRNA will be integrated will Cas protein to end up to the complex containing the Cas protein which the pieces of RNA inside the which the RNA. The interference is between the specific Cas protein and RNA Sequence are going to be merge together. The Differences between three types-

Type 1: The Crispr Sequence is loop then was going to happen phase to bacteriophage infect cell another time so as a told you in bacteriophage infect the cell in first time this pieces was taken from this phase and now this phase comes another time to infect the cell. this pieces of RNA can recognized this pieces of DNA because its complementary.
PAM (Protospacer adjacent motif): the bacterial cell chooses the pieces of viral genome to take it to adjacent of PAM sequences. So the bacterial cell can recognized the PAM sequences and

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[9] Deng L, KenchappaCS, PengX, She Q and GarrettRA 2011 Modulation of CRISPR locus transcription by the repeatbinding protein Cbp1 in Sulfolobus Nucl. Acids Res. 40 2470–80
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[11] Zetsche B et al 2015 Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system Cell 163 759–71

then it take the adjacent sequence in order to add to spacer DNA to order to RNA and CRISPR Complex.
Cas3: the cas3 will chop up and cut the viral genome end of the degraded.so the virus can't in way the cells any more this virus can not the cell any more.

Type 2: This is most important role play in the cas9.The RNA complementary of lower strand they will bind together and then the Cas enzyme itself. The double strand breaks in viral sequences in the double strand mean that Cas9 will break the two strands of DNA exactly at the same place it's called double strand break. The Cas enzyme has two doman. It's called HNH, RuvC and RNaseH-like endonuclease domains.

Type 3: The RNA sequences recognize its complementary the viral genome and they will be bind together cascade cas9 and viral genome will be chopped up.

CRISPR application:
Genome editing is enabled by the development of tools to make precise, targeted changes to the genome of living cells. The modification of targeted genome – zinc-finger nucleases (ZFNs) and transcription-activator like effector nucleases (TALENs) – enable researchers to generate mutations by introducing double-stranded breaks to activate repair pathways. These approaches are costly and time consuming to engineer, limiting their widespread use, particularly for large scale, high-throughput studies. Recently, methods based on a bacterial CRISPR-associated protein-9 nuclease (Cas9) from Streptococcus pyogenic have generated considerable excitement. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes are essential for adaptive immunity in select bacteria and archaea, enabling the organisms to respond to and eliminate invading genetic material. Though, the influential properties of Type II speed the development of eukaryotes. Chiefly gathering of the two type's crRNA and tra-crRNA yields (sgRNA) single guide RNA cemented the pathway of expansion [[12]].Furthermore, Cas9 could healing sever viral infection like hepatitis and human immune-sorbent viruses. A known version of Cas9 could be verified in order to delivering via Adeno-associated virus, another somatic therapeutical purpose based on gene alteration becomes critical and genetic excision which accurate embryos improvement [[13]].

Benefits:

- **Low Cost** - Plasmid DNA is a renewable, cost-effective format.
- **Flexibility** - Cas9 and guide RNA plasmids are suitable for stable or transient transfection.

[12] K. Chylinski, A. Le Rhun, E. Charpentier. The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems, RNA Biol. 10 (5) (2013) 726e737.

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- **Ease-of-use** - Guide RNA oligonucleotide format enables simple retargeting of Cas9 to different loci.
- **DNA Free -** No risk of insertional mutagenesis.
- **High Efficiency Delivery** - Deliver Cas9/gRNA complexes to multiple cell types, including hard to transfect cells such as immune and stem cells.

Discussion: The discussions of the study indicate that CRISPR is very usefully tool in genetic engineering. It's capable to change in desirable gene and help to curing genetic disorder and many more diseases. So it's very important Pandora box of human discovery.

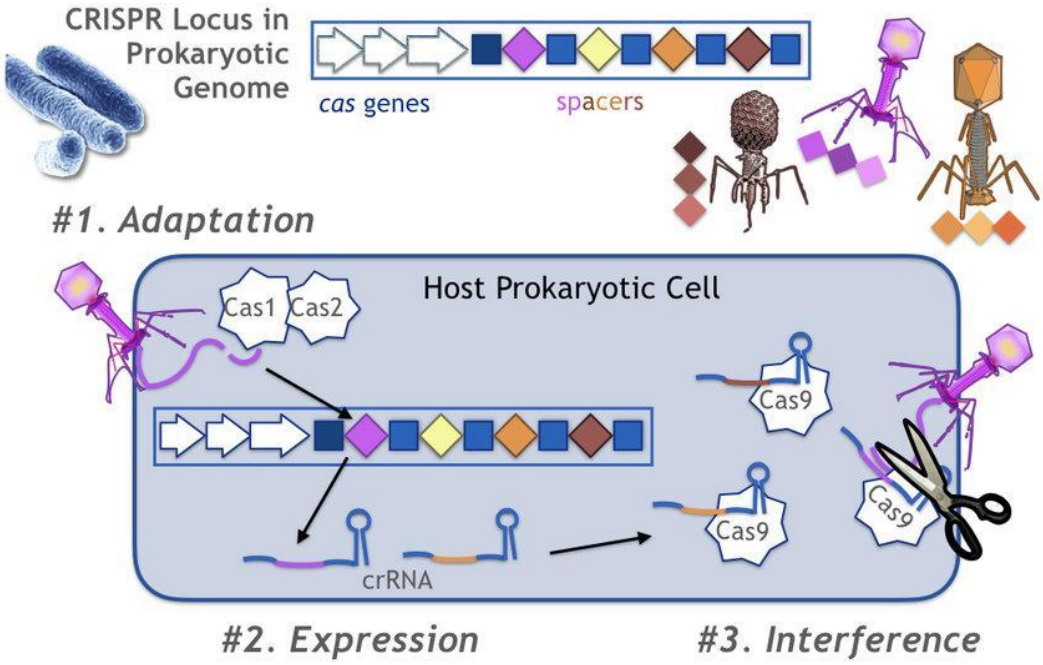


Figure1. Researchers have designated three stages of CRISPR-Cas immunity in a host bacteria or archaea cell, mediated by Cas proteins. New spacers against viral, plasmidic, and other mobile genetic element foes are acquired during adaptation. These spacers are transcribed during the expression stage into guide sequences (crRNAs) that team up with a DNA nuclease Cas protein or complex to protect the host from attack by a matching invader during the interference stage.

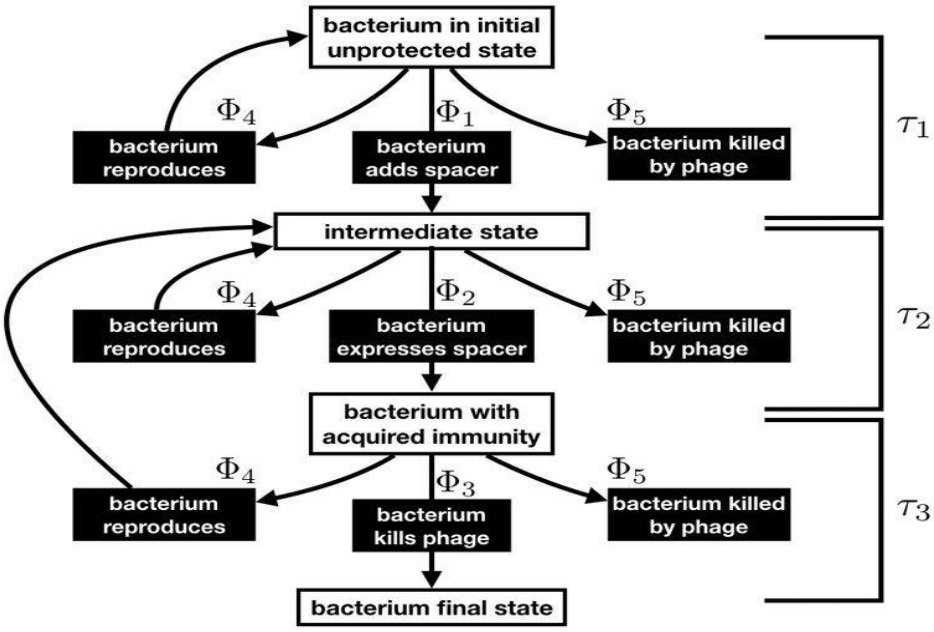
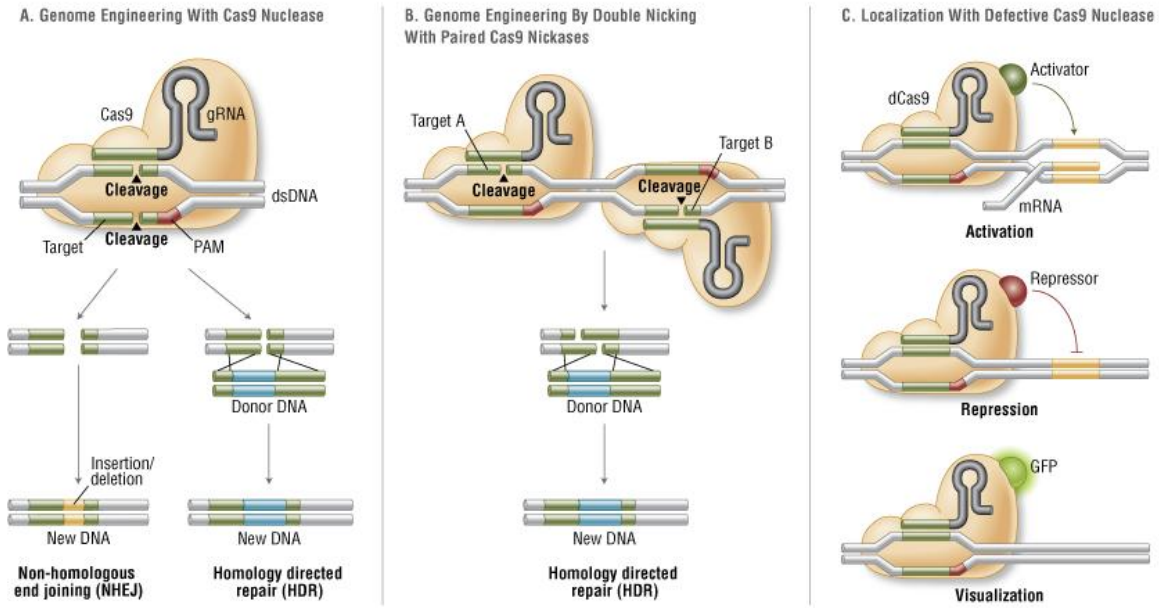


Figure 2. A Markov model for CRISPR adaptation, expression, and interference. The transition event rates Φi depend on the characteristic timescales for adaptation by spacer acquisition τ1, expression τ2, and interference τ3.



[14]

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Figure: 3 CRISPR/Cas9 System Applications

Conclusion: The CRISPR-Cas9 is the key of genetic revaluation era. This tool will be change method to studying the genetic code. It's had to be research and studying in the genome editing and improve many diseases. CRISPR will help to designing the new baby, improved disorder and focus to the targeting gene and CRISPR Specific protein immunity against mobile genetic elements with spacer sequences chronicled in the CRISPR locus. Finally summarized in this review both in modeling and the CRISPR/Cas9 system have emerged as a feasible tool to achieve what has seemed impossible for decades.

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Conflicts of Interest: The authors declare no conflict of interest.

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