J Appl Biotechnol Rep. 2020 June;7(2):81-86

doi 10.30491/JABR.2020.109380

Journal of
Applied Biotechnology
Reports

Review Article

CRISPR Arrays: A Review on Its Mechanism

Sahar Mohamadi[1], Saeed Zaker Bostanabad[1], Reza Mirnejad[2*]

[1]Department of Biology, Islamic Azad University, Parand Branch, Tehran, Iran
[2]Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

Corresponding Author: Reza Mirnejad, PhD, Professor of Bacteriology, Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran. Tel/Fax: +98-21-88039883, Email: rmirnejad@bmsu.ac.ir

Received April 29, 2019; **Accepted** September 10, 2019; **Online Published** June 24, 2020

Abstract
Clustered regularly interspaced short palindromic repeats (CRISPR) which is literally defined as a clustered regularly interspaced short palindromic repeats is an adaptive immune system in bacteria, which enables them to detect and destroy the DNA of the virus. In fact, CRISPR is a defense mechanism in prokaryotic cells, which induces resistance to foreign genetic content, such as that found in the plasmid or phages. The proteins involved in this mechanism are called CRISPR associated proteins (CAS) which have the ability to search, cut and eventually transform phage DNA in a specific way. The CAS is a protein with an enzymatic function which can be called nuclease, given the fact that it plays a special role in the DNA sequence and CRISPR arrays. The CRISPR technology allows changes in DNA, which enables to modify and make any changes to any gene in any living creature, much more accurately and better than all previous methods. In this review, we introduce the mechanisms and benefits of CRISPR in genome editing, briefly reviewing CRISPR programs in gene therapy exploration and CRISPR's ability to produce different types of mutations through different repair mechanisms.
Keywords: CRISPR, CAS Protein, Spacer, Proto-SPACER, Direct Repeats
Citation: Mohamadi S, Zaker Bostanabad S, Mirnejad R. CRISPR arrays: a review on its mechanism. J Appl Biotechnol Rep. 2020;7(2):81-86. doi:10.30491/JABR.2020.109380.

Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR) is an adaptive immune system in bacteria, which enables them to detect and destroy the DNA of the virus.[1,2] The discovery of compatible immune systems like CRISPR in prokaryotes is one of the exciting developments in microbiology in the past decade.

Due to their programmable nature, the CRISPR arrays have been introduced into the public consciousness as genomic editing tools. CRISPR's ability to adapt and protect against attackers that have been previously unused has made it possible to easily plan with custom RNA guides. This in turn has led to the use of CRISPR as an exceptional tool for editing genome directing.[3] The CRISPR array consists of two major elements; the first element is a cassette with direct repeats of DNA, with 21-47 bp length which is interspersed with nonrepetitive sequences and the second element is a set of CAS genes[1,2,4] (Figure 1).

Direct repeats (DRs) are conserved sequences, except the repeat sequence in one end of CRISPR, which is generally not conserved and therefore the DRs are called degenerates. On the other hand, SPACER sequences are totally different. The SPACER is a non-repetitive sequence in the CRISPR array which is created by Proto-SPACERs located in the aggressive genetic elements such as phages and plasmids. It helps to protect the cell from subsequent infection by the same phages, when placed inside the CRISPR of bacterial cell.[5-10] *Proto*-SPACER is an extension in external DNA, which is known as a SPACER in the host genome.[11] The active sites of CRISPR are evolved by adding several new SPACERs exposed to the bacteriophages. However, genome sequencing analysis revealed the involvement of a variety of single nucleotide polymorphisms (SNPs) and INDEL (insert/delete).[12-15] Several bioinformatics studies have been published that show the match of CRISPR SPACER content with the bacteriophage genome.[16-18]

The CRISPR in one end is accompanied by leader sequence before the first DR, which is believed that acts as a promoter for CRISPR transcription in small RNAs.

The CAS proteins (CRISPR-associated) are coded by operons located in the vicinity of the CRISPR sequence and contain the domains that comprise the functional portion of nucleases, helicases, polymers, and polynucleotide binding proteins.[19-21] The CAS genes act with two types of RNAs. In this process, the CAS genes act as scissors and a piece of RNA as an addressing tag and ensure that cutting is done in the right place.[2-4] Most of the genes related to the CRISPR have been found in one part in the CRISPR region,[22] whereas CAS genes are found in different regions of CRISPR. Naturally, this short genetic element (CAS) occurs only through the attack of

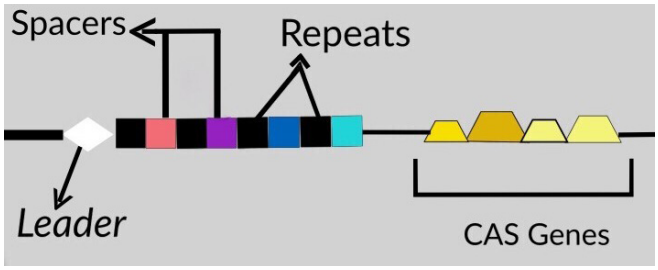

Figure 1. Structure CRISPR array. The CRISPR array consists of duplicate sequences (repeats) that are separated by variable sequences (spacers) from the attack by mobile genetic elements (MGEs). The CAS genes are usually located near the CRISPR locus but can be encrypted elsewhere on the genome.[2]

viruses (not the bacterial genome), and therefore ensures that the CAS does not eliminate its CRISPR position.[23]

Some of the CAS proteins have the ability to detect foreign DNA which attacks the bacteria. CRISPR RNAs (crRNAs) create a new repeat-spacer from proto-SPACERs of external DNA and import it to CRISPR in leader end. The crRNA is hybridized with tracrRNA (and coded by CRISPR system) and this pair of RNA is linked to CAS nucleases. The crRNA/tracrRNA/CAS9 complex is responsible for the diagnosis and separation of external DNA with Proto-SPACER. The CRISPR/CAS is an engineered system composed of convergence between crRNA and part of the tracrRNA sequence.[24] Therefore, when the same exogenous nucleic acid is attached, CRISPR transcript crRNA can be diagnosed and decomposed external nucleic acid. With this method, CRISPR with CAS proteins can block external sequences like phages and plasmids[25,26] (Figure 2). The function of the CAS protein in CRISPR is similar to the RNAi function in eukaryotes.[24]

Diagnosis of the target DNA by CAS enzyme is achieved by a Proto-SPACER adjacent to the sequence motif (PAM) which is located directly in the strand section of non-target DNA. If a correct nucleotide is created, CAS9 degenerate double-stranded DAN at 3-4 nucleotides after PAM site. The PAMs usually have several nucleotide sequences and their sequences differ according to the CRISPR/CAS systems.[11,27] The gRNA gene in addition to CAS, leads to the separation of target DNA locus, which is complementary to the first 20 nucleotides of gRNA (in the 5′ end) and adjusts to PAM sequence.[11,28]

Originally, the CRISPR/CAS system is used for the genotyping purposes[29,30] and epidemiological studies based on the hypervariable nature. It studies the relationship between host and virus in natural systems.[10,31,32] Several studies have shown that CRISPR hypervariable locus can be used for the bacterial genotype separation and presents an excellent framework for the pathogen detection and evolutionary studies. In fact, this technique has been used to describe various pathogens.[33-35] Hypervariable locus is widely distributed among species based on the number of CRISPR/CAS systems and CAS genes, DRs and SPACER content.[10]

Classification of CRISPR

Generally, three types of CRISPR/CAS systems (I, II, III) are created based on the sequence of CAS genes.[36]

The CAS1 and CAS2 are available in all CRISPR/CAS systems. They are expected to be active and appear to be involved in the information processing system in the integration of the SPACER in the adaptation phase.[36] Among CRISPR/CAS systems in *Streptococcus thermophilus*, CRISPR1 and CRISPR3 have the ability to read and gain new SPACERs which are exposed to bacteriophage or plasmid.[9,37] Although, the occurrence of each CRISPR in this species can be widely different.[12,14,30]

Several studies have shown that, CRISPR region was classified to 8 subsets which are widely distributed in bacteria and archaea (*Escherichia, Yersinia, Neisseria, Desulfovibrio, Thermotoga, Haloarcula, Apern* and *Mycobacterium*).[23]

These eight distinct subsystems of the CRISPR/CAS system (CASS1-CASS8) are defined by the composition and structure of the CAS protein and the CAS1 phylogene.[23,24]

The CRISPR subsets in each set were different in DR, SPACER and CAS gene section. For example, in *Yersinia* and *Escherichia* the DR, SPACER, and CAS were different. Only one CAS1 gene was found in all subsets of the CRISPR region.[23]

Each of these 8 subsets have regions related to the CRISPR/CAS, which is called CSY and Y indicates the *Yersinia* subset. The CSE, for example, is the CRISPR region in *Escherichia* subset.[23]

Immunologic Function of CRISPR

The discovery of CRISPR/CAS compatible with immune systems in prokaryotes has been one of the most exciting developments in microbiology over the last decade.[22] The ability of mobile genetic elements (MGEs) to change the

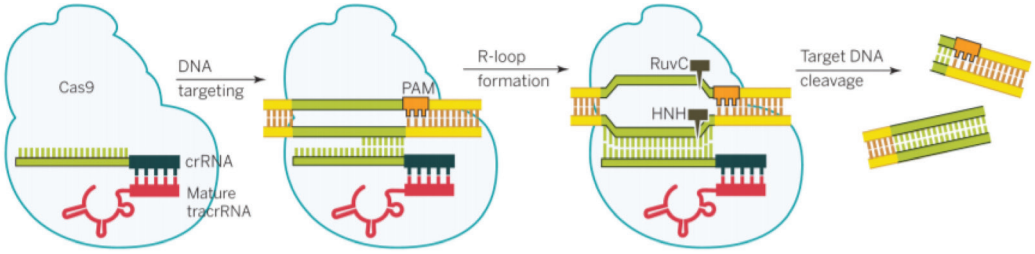

Figure 2. Details of Natural DNA Occurrence With Duplex tracrRNA, CRRNA.[26] crRNA/tracrRNA/CAS complex is responsible for the diagnosis and separation of external DNA with Proto-SPACER. The CAS gene operon with tracrRNA and the CRISPR array and the natural antiviral defense pathway include CAS communication with tracrRNA/crRNA, RNA co-processing by ribonuclease III, R-loop formation, and DNA cleavage. When the same exogenous nucleic acid is attached, CRISPR transcript crRNA can be diagnosed and decomposed external nucleic acid. With this method, CRISPR with CAS proteins can block external sequences like phages and plasmids.

structure (genotype) or the bacterial phenotype is well-known.[38] The CRISPR in prokaryotes is an effective defense system against MGEs especially bacteriophages, plasmids and transposons.[39] There is evidence that this system plays a role in other processes, such as genetic adjustment, DNA repair and genome development.[39-44] Recognizing the role of CRISPR/CAS in these processes will improve our understanding of the development and maintenance of the CRISPR/CAS system in the prokaryotic genome.[44-46]

The CRISPR locus, along with the CAS genes, forms the CRISPR/CAS immune system, which is present in 46% of bacteria and about 90% of archaea.[36,47] Bacteria and archaea use different defense systems to protect against phages and other parasites. Although many defense systems, such as restriction systems, provide the innate immune system, almost half of the bacteria and most archaea have compatible immunity that is coded by the CRISPR/CAS system.[8,48] In previous studies, the effect of CRISPR in resistance to bacteriophages and lytic plasmids have been proven.[12,49] The first direct evidence for the use of these systems in defense was obtained from the study conducted on *Streptococcus thermophilus* which showed resistance to the phage infection by combining proto-SPACER sequences with CRISPR locus.[12] However, CRISPR resistance to bacteriophage and plasmids has been observed in *Streptococcus thermophilus* and *Staphylococcus epidermidis*, respectively.[12,49,50]

The CRISPR/CAS system in terms of immunity and attack on genetic elements was divided to a three-step process: adaptation, expression, and interference into two systems, which include the information processing system and the system of execution. A data processing system which is highly protected includes an adaptation phase and an execution system that includes the expression and interference phases (Figure 3). This is while the proteins (CAS1 and CAS2) associated with the information processing system are likely to be highly protected. These proteins vary widely between different organisms.[8,48,51,52]

In the adaptation phase, where the immunity is obtained by integrating new SPACERs from invasive elements into the CRISPR bacterial points, a Proto-SPACER with a specific length of approximately 30 bp, placed on the CRISPR leader side is created.[9,12] Each adaptation event is associated with a protected sequence (DR) and thus creates a new unit.[9,53]

The second stage in the immunity of CRISPR/CAS is the expression by which the initial transcript is produced from the CRISPR locus, and then the short and mature crRNAs are processed.[36,53] The third stage is interference, during which crRNAs trigger CAS proteins to decompose a particular sequence of phage supplemental nucleic acids.[14,36,40,53]

Proto-SPACER coded with crRNA, binds CAS9 to target DNA sequences near the PAMs sequence for decomposition. The SPACER sequence that is connected to the CRISPR position is not decomposed because there is no PAM sequence next to them.[28]

The CRISPR RNA transcript is processed along with the CAS proteins and through a mechanism similar to RNAi, which detects and destroys the target DNA sequence (RNAi is a process that inhibits gene expression by RNA molecules).[28]

Genome Engineering by CRISPR Sequence

After the scientists understood the ability of CRISPR, genome engineering was quickly expanded. While mice are traditionally the most popular model of the organism for the transgenic testing, CRISPR programs are also shown in a wide range of cells and living organisms, including human embryos.[54] Recently, the ability to reprogram the CAS9 endonuclease using synthetic small RNA guidelines has altered the genome.[54,55]

The CRISPR/CAS II compatible immune system in prokaryotic cells showed that the guide RNA can help to cut a certain sequence of DNA. During the studies, two different types of CRISPR/CAS were designed and it was shown that it

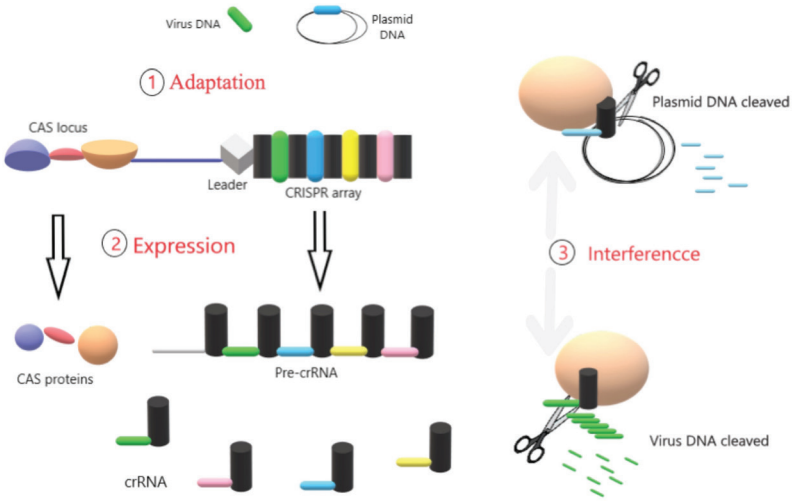

Figure 3. The CRISPR Adaptive Immune System Features. Step 1: CRISPR adaptation. Protospacers of DNA obtained two strands of a virus or plasmid at the end of the leader of a CRISPR array in the host DNA. The CRISPR array consists of unique spacers between repetitions. Adaptation occurs by a process that requires minimal CAS encryption at the CAS locus, which is usually adjacent to the CRISPR array. Step 2: The expression of CRISPR. Pre-crRNA is transcribed by RNA polymerase from the leader region and is further divided into smaller crRNAs. Step 3: CRISPR interference. crRNA containing a spacer that strongly matches an input nucleic acid (plasmid or virus) initiates a fission event (indicated by scissors). Cas proteins are needed for this process.[27]

is possible to direct CAS with short RNAs to produce a precise cleavage in intracellular genomic sites in human and mouse cells.[54]

The magic of CRISPR is in its ability for both DNA strands. The cells should repair dubele-strand breaks (DSBs), which are the result of CAS decomposition or they are exposed to death. All of the CRISPR engineering's is due to the inherent ability of the cell to repair itself.[56] The CRISPR-II system of *Streptococcus pyogenes* was compatible to induce a specific sequence of DSB and genome re-engineering.[56]
There are two repair paths, each of which can be used for engineering: one is NHEJ and the second is HDR

Non-homologous End Joining

If the goal is only to disrupt the function of the gene, then no protein will be produced. As a result, the non-homologous end joining (NHEJ) repair mechanism is used.[56]

The NHEJ is a mechanism for connecting two broken strings. It is subjected to error and may add or remove nucleotides in this process. If the number of added or deleted nucleotides make changes in the gene reading frame, it causes a framing mutation which may eventually end the function of the gene.[56,57]

Homology-Directed Repair

The homology-directed repair (HDR)is a cellular mechanism that uses a homologous DNA template to eliminate dual strands in DNA.[57] If the objective of the test is to replace the targeted genetic element with a different sequence in the cell (e.g. gene insertion, basic redundancy, etc.), HDR can be used. For this purpose, a homologous DNA model with the desired sequence with CRISPR components in the cell should be introduced.[57]

A certain number of the cells use this mechanism to repair the broken sequences through the homologous recombinant sequences, thereby add the desired changes to the genome.[54,57,58]

Parvalbumin (PVALB) genomic locus in human and tyrosine hydroxylase (TH) in mice were tested by precrRNA and simulated RNAs. The CRISPR/CAS effective changes were investigated in TH of three mice and PVALB in human. It was discovered that CRISPR/CAS could widely be used in modifying different loci in different organisms. For the effective engineering of the genome, there is a need for a specific genomic target nucleus with high accuracy and efficiency. To investigate the modification of a particular RNA genome, single nucleotide mismatch between the SPACER and target Proto-SPACER were analyzed in mammals. It was shown that, the mismatch up to 11 bp in the 5′ sequence of PAM was completely deleted by CAS9, where the target proto-SPACERs were preserved against mutated SPACER.[54,56] Currently, CRISPR could be used for the gene engineering in a wide range of organisms.[59]

A virus was designed as a carrier of CRISPR components to the mouse. The virus was infiltrated in the mice, allowing the CRISPR system to engineer the mutations and create a model for the human lung cancer.[60] The *ALK* and *EML4* genes, which are expressed by CAS9 induction, were inserted into PX330 plasmid, and after PCR, it was shown that the sequence between the two transmitted genes, which is a large area, was also eliminated and this deletion occurred in the entire cell population of the transmitted genes. A similar strategy was also modeled for Npm1-ALk and transferred to the chromosome, which is commonly seen in large cell lymphomas. These results indicated that the CRISPR system can be injected into eukaryotic cells in order to remove, magnify, and transfer chromosomes.[60] To target the ALK and EML4 loci by CAS9 and sgRNA in mice lung, the CAS9 and sgRNA were inserted into the vector and recombinant adenovirus (Ad-EA) was constructed. The adenovirus vectors are effective models as they effectively infect the mouse lung epithelium, but they do not integrate into the host genome.[61] Mice embryo fibroblast infection with Ad-EA led to CAS9 and sgRANs expression and rapid production of EmL4-ALk reverse template. Overall, these results indicated that CRISPR technology has the ability to reconstitute the oncogenic chromosomes in mice.[61]

Mice with lung cancer were induced by EmL4-ALk. They showed complete inhibition of molecular and biological properties of human ALK+NSCLCS like specific sensitivity to ALK inhibitor. This model offers unique opportunities to detect molecular mechanisms through which EmL4-ALk prevents tumor formation. It is suitable for testing the efficacy of targeted therapies and evaluating in vivo drug resistance mechanisms.[60,61] The CAS9 can be used for the DNA cutting in different in vitro and in vivo situations.[62-64] The CAS9 which acts on single strands, instead of the double strand on the target DNA site is useful for the genome engendering.[23,24]

With reducing the genetic diversity of plants, the products are limited and will prevent production for future demand, thus, there is an urgent need for the effective performance improvement strategies with new genome engineering techniques such as the CRISPR/CAS9 system, which can improve the existing critical performance or produce new and better products.[65]

Conclusions

The CRISPR/CAS system is present in the genome of most of the prokaryotes which act as an immune system against phages and plasmids. The system is rapidly developing and adapting to the potential application in genotyping epidemiological and evolutionary studies, and genomic engineering. By reprogramming the system using synthetic small guide RNA strands and the ability of CAS9 to cut different sequences of DNA in a variety of cells and organisms, it might be possible to modify the genome by applying changes in the genome of the eukaryotes using vectors.

Authors' Contributions

All authors contributed equally to this study.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

References

1. Marraffini LA. CRISPR-Cas immunity against phages: its effects on the evolution and survival of bacterial pathogens. PLoS Pathog.

2013;9(12):e1003765. doi:10.1371/journal.ppat.1003765.
2. Westra ER, Buckling A, Fineran PC. CRISPR-Cas systems: beyond adaptive immunity. Nat Rev Microbiol. 2014;12(5):317-326. doi:10.1038/nrmicro3241.
3. Hynes AP, Labrie SJ, Moineau S. Programming native CRISPR arrays for the generation of targeted immunity. mBio. 2016;7(3). doi:10.1128/mBio.00202-16.
4. Li R, Fang L, Tan S, et al. Type I CRISPR-Cas targets endogenous genes and regulates virulence to evade mammalian host immunity. Cell Res. 2016;26(12):1273-1287. doi:10.1038/cr.2016.135.
5. Bolotin A, Quinquis B, Sorokin A, Ehrlich SD. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology. 2005;151(Pt 8):2551-2561. doi:10.1099/mic.0.28048-0.
6. Pourcel C, Salvignol G, Vergnaud G. CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology. 2005;151(Pt 3):653-663. doi:10.1099/mic.0.27437-0.
7. Mojica FJ, Díez-Villaseñor C, García-Martínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J Mol Evol. 2005;60(2):174-182. doi:10.1007/s00239-004-0046-3.
8. Deveau H, Garneau JE, Moineau S. CRISPR/Cas system and its role in phage-bacteria interactions. Annu Rev Microbiol. 2010;64:475-493.doi:10.1146/annurev.micro.112408.134123.
9. Garneau JE, Dupuis M, Villion M, et al. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature. 2010;468(7320):67-71. doi:10.1038/nature09523.
10. Barrangou R, Horvath P. CRISPR: new horizons in phage resistance and strain identification. Annu Rev Food Sci Technol. 2012;3:143-162.doi:10.1146/annurev-food-022811-101134.
11. Barrangou R. CRISPR-Cas systems and RNA-guided interference. Wiley Interdiscip Rev RNA. 2013;4(3):267-278. doi:10.1002/wrna.1159.
12. Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;315(5819):1709-1712. doi:10.1126/science.1138140.
13. Deveau H, Barrangou R, Garneau JE, et al. Phage response to CRISPR-encoded resistance in Streptococcus thermophilus. J Bacteriol. 2008;190(4):1390-1400. doi:10.1128/jb.01412-07.
14. Horvath P, Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea. Science. 2010;327(5962):167-170. doi:10.1126/science.1179555.
15. Barrangou R, Coûté-Monvoisin AC, Stahl B, et al. Genomic impact of CRISPR immunization against bacteriophages. Biochem Soc Trans. 2013;41(6):1383-1391. doi:10.1042/bst20130160.
16. Vestergaard G, Shah SA, Bize A, et al. Stygiolobus rod-shaped virus and the interplay of crenarchaeal rudiviruses with the CRISPR antiviral system. J Bacteriol. 2008;190(20):6837-6845. doi:10.1128/jb.00795-08.
17. Hale CR, Zhao P, Olson S, et al. RNA-guided RNA cleavage by a CRISPR RNA-Cas protein complex. Cell. 2009;139(5):945-956. doi:10.1016/j.cell.2009.07.040.
18. Díez-Villaseñor C, Almendros C, García-Martínez J, Mojica FJ. Diversity of CRISPR loci in Escherichia coli. Microbiology. 2010;156(Pt 5):1351-1361. doi:10.1099/mic.0.036046-0.
19. Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell. 2009;136(4):642-655. doi:10.1016/j.cell.2009.01.035.
20. Terns MP, Terns RM. CRISPR-based adaptive immune systems. Curr Opin Microbiol. 2011;14(3):321-327. doi:10.1016/j.mib.2011.03.005.
21. Bondy-Denomy J, Pawluk A, Maxwell KL, Davidson AR. Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system. Nature. 2013;493(7432):429-432. doi:10.1038/nature11723.
22. Zegans ME, Wagner JC, Cady KC, Murphy DM, Hammond JH, O'Toole GA. Interaction between bacteriophage DMS3 and host CRISPR region inhibits group behaviors of Pseudomonas aeruginosa. J Bacteriol. 2009;191(1):210-219. doi:10.1128/jb.00797-08.
23. Haft DH, Selengut J, Mongodin EF, Nelson KE. A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes. PLoS Comput Biol. 2005;1(6):e60. doi:10.1371/journal.pcbi.0010060.
24. Makarova KS, Grishin NV, Shabalina SA, Wolf YI, Koonin EV. 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. 2006;1:7. doi:10.1186/1745-6150-1-7.
25. Cady KC, Bondy-Denomy J, Heussler GE, Davidson AR, O'Toole GA. The CRISPR/Cas adaptive immune system of Pseudomonas aeruginosa mediates resistance to naturally occurring and engineered phages. J Bacteriol. 2012;194(21):5728-5738. doi:10.1128/jb.01184-12.
26. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213):1258096. doi:10.1126/science.1258096.
27. Bhaya D, Davison M, Barrangou R. CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. Annu Rev Genet. 2011;45:273-297. doi:10.1146/annurev-genet-110410-132430.
28. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol. 2014;32(4):347-355. doi:10.1038/nbt.2842.
29. Makarova K, Slesarev A, Wolf Y, et al. Comparative genomics of the lactic acid bacteria. Proc Natl Acad Sci U S A. 2006;103(42):15611-15616.doi:10.1073/pnas.0607117103.
30. Horvath P, Romero DA, Coûté-Monvoisin AC, et al. Diversity, activity, and evolution of CRISPR loci in Streptococcus thermophilus. J Bacteriol. 2008;190(4):1401-1412. doi:10.1128/jb.01415-07.
31. Tyson GW, Banfield JF. Rapidly evolving CRISPRs implicated in acquired resistance of microorganisms to viruses. Environ Microbiol. 2008;10(1):200-207. doi:10.1111/j.1462-2920.2007.01444.x.
32. Pride DT, Sun CL, Salzman J, et al. Analysis of streptococcal CRISPRs from human saliva reveals substantial sequence diversity within and between subjects over time. Genome Res. 2011;21(1):126-136. doi:10.1101/gr.111732.110.
33. Grissa I, Vergnaud G, Pourcel C. Clustered regularly interspaced short palindromic repeats (CRISPRs) for the genotyping of bacterial pathogens. In: Caugant DA, ed. Molecular Epidemiology of Microorganisms. Springer; 2009. p. 105-116.
34. Pourcel C, Drevet C. Occurrence, diversity of CRISPR-Cas systems and genotyping implications. In: Barrangou R, van der Oost J, eds. CRISPR-Cas Systems. Berlin, Heidelberg: Springer; 2013. p. 33-59. doi:10.1007/978-3-642-34657-6_2.
35. Briner AE, Barrangou R. Lactobacillus buchneri genotyping on the basis of clustered regularly interspaced short palindromic repeat (CRISPR) locus diversity. Appl Environ Microbiol. 2014;80(3):994-1001. doi:10.1128/aem.03015-13.
36. Makarova KS, Haft DH, Barrangou R, et al. Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiol. 2011;9(6):467-477. doi:10.1038/nrmicro2577.
37. Horvath P, Coûté-Monvoisin AC, Romero DA, Boyaval P, Fremaux C, Barrangou R. Comparative analysis of CRISPR loci in lactic acid bacteria genomes. Int J Food Microbiol. 2009;131(1):62-70. doi:10.1016/j.ijfoodmicro.2008.05.030.
38. Brüssow H, Canchaya C, Hardt WD. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiol Mol Biol Rev. 2004;68(3):560-602, table of contents. doi:10.1128/mmbr.68.3.560-602.2004.
39. Sampson TR, Weiss DS. CRISPR-Cas systems: new players in gene regulation and bacterial physiology. Front Cell Infect Microbiol.

2014;4:37. doi:10.3389/fcimb.2014.00037.
40. Wiedenheft B, Sternberg SH, Doudna JA. RNA-guided genetic silencing systems in bacteria and archaea. Nature. 2012;482(7385):331-338. doi:10.1038/nature10886.
41. Edgar R, Qimron U. The Escherichia coli CRISPR system protects from λ lysogenization, lysogens, and prophage induction. J Bacteriol. 2010;192(23):6291-6294. doi:10.1128/jb.00644-10.
42. Barrangou R, Horvath P. The CRISPR system protects microbes against phages, plasmids. Microbe. 2009;4(5):224-230. doi:10.1128/microbe.4.224.1.
43. Fabre L, Zhang J, Guigon G, et al. CRISPR typing and subtyping for improved laboratory surveillance of Salmonella infections. PLoS One. 2012;7(5):e36995. doi:10.1371/journal.pone.0036995.
44. Louwen R, Horst-Kreft D, de Boer AG, et al. A novel link between Campylobacter jejuni bacteriophage defence, virulence and Guillain-Barré syndrome. Eur J Clin Microbiol Infect Dis. 2013;32(2):207-226. doi:10.1007/s10096-012-1733-4.
45. Westra ER, Swarts DC, Staals RH, Jore MM, Brouns SJ, van der Oost J. The CRISPRs, they are a-changin': how prokaryotes generate adaptive immunity. Annu Rev Genet. 2012;46:311-339. doi:10.1146/annurev-genet-110711-155447.
46. Labrie SJ, Samson JE, Moineau S. Bacteriophage resistance mechanisms. Nat Rev Microbiol. 2010;8(5):317-327. doi:10.1038/nrmicro2315.
47. Grissa I, Vergnaud G, Pourcel C. The CRISPRdb database and tools to display CRISPRs and to generate dictionaries of spacers and repeats. BMC Bioinformatics. 2007;8:172. doi:10.1186/1471-2105-8-172.
48. van der Oost J, Jore MM, Westra ER, Lundgren M, Brouns SJ. CRISPR-based adaptive and heritable immunity in prokaryotes. Trends Biochem Sci. 2009;34(8):401-407. doi:10.1016/j.tibs.2009.05.002.
49. Marraffini LA, Sontheimer EJ. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science. 2008;322(5909):1843-1845. doi:10.1126/science.1165771.
50. Brouns SJ, Jore MM, Lundgren M, et al. Small CRISPR RNAs guide antiviral defense in prokaryotes. Science. 2008;321(5891):960-964. doi:10.1126/science.1159689.
51. Al-Attar S, Westra ER, van der Oost J, Brouns SJ. Clustered regularly interspaced short palindromic repeats (CRISPRs): the hallmark of an ingenious antiviral defense mechanism in prokaryotes. Biol Chem. 2011;392(4):277-289. doi:10.1515/bc.2011.042.
52. Karginov FV, Hannon GJ. The CRISPR system: small RNA-guided defense in bacteria and archaea. Mol Cell. 2010;37(1):7-19. doi:10.1016/j.molcel.2009.12.033.
53. Mojica FJM, Díez-Villaseñor C, García-Martínez J, Almendros C. Short motif sequences determine the targets of the prokaryotic CRISPR defence system. Microbiology. 2009;155(Pt 3):733-740. doi:10.1099/mic.0.023960-0.
54. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339(6121):819-823. doi:10.1126/science.1231143.
55. Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nat Biotechnol. 2013;31(3):233-239. doi:10.1038/nbt.2508.
56. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816-821. doi:10.1126/science.1225829.
57. Khatodia S, Bhatotia K, Passricha N, Khurana SM, Tuteja N. The CRISPR/Cas genome-editing tool: application in improvement of crops. Front Plant Sci. 2016;7:506. doi:10.3389/fpls.2016.00506.
58. Wu Y, Liang D, Wang Y, et al. Correction of a genetic disease in mouse via use of CRISPR-Cas9. Cell Stem Cell. 2013;13(6):659-662. doi:10.1016/j.stem.2013.10.016.
59. Vyas VK, Barrasa MI, Fink GR. A Candida albicans CRISPR system permits genetic engineering of essential genes and gene families. Sci Adv. 2015;1(3):e1500248. doi:10.1126/sciadv.1500248.
60. Maddalo D, Manchado E, Concepcion CP, et al. In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. Nature. 2014;516(7531):423-427. doi:10.1038/nature13902.
61. DuPage M, Dooley AL, Jacks T. Conditional mouse lung cancer models using adenoviral or lentiviral delivery of Cre recombinase. Nat Protoc. 2009;4(7):1064-1072. doi:10.1038/nprot.2009.95.
62. Mali P, Yang L, Esvelt KM, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339(6121):823-826. doi:10.1126/science.1232033.
63. Cho SW, Kim S, Kim JM, Kim JS. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nat Biotechnol. 2013;31(3):230-232. doi:10.1038/nbt.2507.
64. Hwang WY, Fu Y, Reyon D, et al. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol. 2013;31(3):227-229. doi:10.1038/nbt.2501.
65. Chen K, Gao C. Targeted genome modification technologies and their applications in crop improvements. Plant Cell Rep. 2014;33(4):575-583. doi:10.1007/s00299-013-1539-6.