https://doi.org/10.31489/2025BMG1/65-73

UDC 577.212.3 Received: 31.05.2024 | Accepted: 13.12.2024

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Gene editing by CRISPR-Cas–biotechnological applications

The CRISPR-Cas system is a powerful genome editing tool that enables precise and targeted changes in DNA nucleotide sequence and gene function. It has many applications in modern biotechnology, including correcting genetic disorders, treating and preventing disease, as well as improving animal breeds and crop growth and resistance. The proposed review observed how CRISPR-Cas technology has evolved from a natural antiviral defense mechanism in bacteria, to a cutting-edge genetic engineering technique. Initially, it was discovered that bacteria use specialized RNA and Cas proteins as defense against viral attacks. It turned out that components of the CRISPR system could be transferred into cells of other organisms to manipulate genes, a process called "gene editing". In genome editing, genetic instructions are altered, resulting in changes in the activity of encoded proteins and cellular processes. By inserting cuts into the coding part of DNA and DNA repair mechanisms, desired DNA fragments can be inserted for targeted changes. CRISPR technology can effectively correct human genetic defects. Examples include cystic fibrosis, sickle cell anemia, cataracts, etc. These studies have paved the way for therapeutic applications in humans. CRISPR has been tested to treat cancer and an inherited disease that causes blindness, prevent Lyme disease and the transmission of malaria transmission from viral vectors to humans, as well as the method of ridding infected cells of human immunodeficiency virus was tested on animal models. The review provides the most significant examples of application of CRISPR editing of target regions of genomes of various organisms as one of the key technologies of biotechnology.

Keywords: CRISPR, Cas, genome editing, biotechnology.

One of the exciting discoveries in modern molecular biology — honored with the Nobel Prize in 2020 — is the CRISPR-Cas genome editing technology [1]. This technology allows the targeted addition, deletion, or modification of DNA sequences and is actively used in genetics and medicine, offering advantages such as rapidity, affordability, high accuracy, and less labor-intensive than other methods.

Originally, the term CRISPR-Cas referred to a natural mechanism for recognizing and destroying viral nucleic acids that penetrate bacterial cells.

If the bacteria was able to survive after virus infection, fragments of the remaining viral DNA are incorporated into a special region of bacterial DNA, adding to the collection of viral fingerprints called CRISPR [2]. Upon re-infection, the bacteria rapidly synthesize RNA that recognizes the DNA regions of the attacking virus and forms a duplex structure. A special nuclease Cas, accompanying complementary RNA destroys viral DNA, preventing the synthesis of new viral particles [3].

In 1987, a team of scientists at Osaka University studied the alkaline phosphatase gene, which is responsible for isoenzyme conversion of alkaline phosphatase (iap) in E. coli strain K-12 [4]. What was surprising was the region that did not encode anything. Bacterial DNA is sparingly organized and usually contains no extra sequences. This site contained unusual repetitive DNA sequences separated by blocks of about 30 nucleotides of different composition, called spacers. Later, similar regions of repeats and spacers would be found in a large number of bacteria and archaebacteria and called CRISPR. The functions of these DNA regions remained a mystery [5]. In 2000, Francisco Mojica's group [6] showed that the spacer regions of the DNA blocks matched the DNA sequences of bacteriophages and viruses. It turned out that viruses could not destroy bacteria that contained such spacer blocks, and it was suggested that these sequences represented the defense system of prokaryotes. Francisco Mojica was the first to suggest the acronym CRISPR. However, the mechanism of this molecular genetic system remained unclear. Jansen et al. discovered [7] that next to CRISPR sequences there are genes called cas (CRISPR-ASsociated), the function of which remained unclear. In 2005, genomic studies found matches between spacer DNA and many phage DNAs, meaning CRISPR clusters play a role in adaptive immunity [8, 9]. Philip Horvath's group [10] discovered in 2007 CRISPR loci in Streptococcus thermophilus cultures used for yogurt and cheese production. It turned out that bacteria having specific viral sequences in their CRISPR sites were resistant to viruses. When such spacer sites are removed from bacterial DNA, the bacteria become susceptible to the virus again. They also studied cas genes and showed the key role of Cas protein in the cleavage of foreign DNA [11]. Cas protein genes are located in close proximity to the CRISPR site. These proteins possess endonuclease, exonuclease, helicase structure and nucleic acid binding sites. They are able to unfold, unwind and cleave DNA [12, 13].

When CRISPR systems were discovered, they were thought to be a novel mechanism for error correction and DNA repair in thermophilic archaea and bacteria [14].

The role of Cas proteins as specific nucleases was shown in the work of Makarova et al. who performed a comparative genomic analysis of Cas genes [15]. While some Cas proteins cleave DNA, others cleave RNA. For example, Cas9 enzyme cleaves DNA, while Cas13 enzyme cleaves RNA [16]. A prerequisite for CRISPR-Cas9 systems (the most widely used) are “protospacer adjacent motifs (PAMs)”. PAMs are short sequences of 2–6 base pairs in the viral genome, next to protospacer DNA blocks targeted by Cas9 nucleases. If Cas9 nucleases recognize the PAM site they cleave the DNA. Recognition of PAM sites by Cas9 nucleases ensures that only foreign viral nucleic acids are cleaved [17] and prevents cutting of their own spacers stored in the CRISPR array.

Classification of CRISPR-Cas systems was performed in Makarov et al. [18] CRISPR-Cas systems are divided into two classes based on the differences of Cas nucleases and are categorized into six types. Class 1 CRISPR-Cas systems function with multi-Cas complexes including Cas3, Cas10 and DinG endonucleases, constituting types 1, 3 and 4, respectively. CRISPR-Cas class 2 systems utilize a single Cas protein and one Cas protein and include types 2, 5, and 4, which utilize Cas9, Cas12–Cas14, and Cas13 nucleases. Type 1, 2, and 5 systems primarily recognize DNA, type 3 recognizes both RNA and DNA, and type 4 is exclusively involved in RNA regulation [19]. The type 2 system is the most studied and has the greatest potential for genome editing in eukaryotic organisms. The Cas9 endonuclease plays a central role in the type 2 CRISPR-Cas system [20]. During viral infection, Cas9 nuclease is activated in the presence of two RNAs: a short RNA (cr -RNA) transcribed from the CRISPR array and an additional non-coding RNA, trans-activating CRISPR RNA (trans-activating CRISPR RNA, tracrRNA) complementary to CRISPR repeats [21]. Jinek et al. [22] constructed a type 2 CRISPR-Cas system based on the Cas9 nuclease from Streptococcus pyogenes and showed that cr RNA and tracr RNA can be spliced together to form a chimeric single-stranded guide RNA (sg RNA).

Such chimeric RNA and Cas9 nuclease can be targeted to DNA from any organisms for precise editing. The idea of adapting the bacterial CRISPR-Cas9 system to edit the genome of humans and other organisms was first proposed by Jennifer Doudna and Emmanuelle Charpentier in 2012 [23], who were awarded the Nobel Prize in 2020. They proposed to combine crRNA (containing the complementary sequence of target DNA) and tracrRNA molecules to obtain achimeric guide RNA (sgRNA). The resulting sgRNA and the Cas9 enzyme are introduced into the cell, and the cell’s genome DNA is scanned for PAM sequences and sites complementary to the sgRNA, after which the DNA is cleaved at the target site. This pioneering work has turned CRISPR-Cas9 into a powerful tool for genome editing.

The advantages of the CRISPR-Cas9 system are simple assembly of the system before use, high efficiency and applicability for genome editing of a wide variety of organisms. To edit any DNA under study, an sgRNA is created that carries a complementary sequence of 20 nucleotides to guide to the desired site. The Cas9 nuclease cuts the DNA between the 17th and 18th nucleotide from the 5'-end of the spacer. It is possible to simultaneously edit several genes if different sgRNAs are used. The simplicity, speed and efficiency of the CRISPR-Cas9 method compared to other genome editing methods have aroused great interest in the scientific community [24]. The CRISPR-Cas9 system is capable of recognizing the desired site in the extended genomic DNA in cells of organisms with high specificity, allowing the addition, deletion, modification and even silencing of certain sites in the genome [25], which is of great importance for biotechnology and medicine.

The CRISPR genome editing method can be successfully applied not only with the Cas9 enzyme, but also with other enzymes such as the Cas13 enzyme discovered in 2016 [26]. The CRISPR-Cas9 technology has advantages over other genome editing technologies such as ZFN and TALEN. The most important one is that a small region of the RNA molecule, sgRNA, complementary to twenty nucleotides of the target DNA is responsible for recognizing the target nucleotide sequence in it [27]. Cong et al. [28] were among the first to successfully apply Cas9-based genome editing.

Applications of CRISPR technology

1. Medical research and therapy: studying the genetic basis of diseases and developing new gene therapies, e.g., for the treatment of hereditary and cancer diseases.
2. Agriculture and food technology: creating crops and breeds with improved characteristics such as resistance to pests and diseases, increased yield and productivity, and better adaptation to climate change.

3. Diagnostics: developing new, more sensitive and accurate methods for diagnosing diseases, including infectious diseases, as demonstrated during the COVID-19 pandemic.

Medical research and therapeutics

CRISPR-Cas technology has the potential to dramatically improve the situation with human monogenic diseases, of which there are more than 10,000 [29, 30]. It has been shown that CRISPR can be used to efficiently correct mutations in the gene that causes inherited heart disease in human embryos. The mutant gene (MYBPC3) was replaced with a "corrected" copy of the gene without the mutation. As a result, up to 72 % of embryos were spared from the harmful mutation [31]. In the case of the monogenic inherited disease sickle cell anemia, CRISPR-Cas9 technology is used to adjust the gene [32].

Cystic fibrosis (CF) is one of the most common genetic diseases caused by mutations in CF transmembrane conductance regulator (CFTR). CRISPR-Cas9 technology has been used to correct mutations in the CFTR gene, and encouraging results have been obtained [33].

In January 2024, the FDA approved CASGEVY therapy for genome editing with CRISPR-Cas9.

CASGEVY is a therapy used to treat people aged 12 years and older for the treatment of:

- sickle cell anemia;
- beta-thalassemia (β-thalassemia).

CASGEVY is based on using edited stem cells from a patient's blood to increase fetal hemoglobin synthesis and red blood cell activity. It helps patients with sickle cell anemia and beta-thalassemia [34, 35].

Hemophilia B (HB) is an inherited disease arising from a mutation in the factor IX (FIX) gene, which leads to clotting disorders. Animal experiments, showed the possibility of treating hemophilia B by adding regulatory genes to correct mutations in the FIX gene using CRISPR-Cas9 technology [36].

In addition to the treatment of monogenic diseases, CRISPR-Cas systems have been used to potentially treat viral infections such as human immunodeficiency virus, hepatitis viruses, and oncogenic viruses, as well as non-viral infectious diseases caused by bacteria, fungi, and parasites [37].

The ability of the CRISPR-Cas9 system to eliminate the integrated ssDNA of hepatitis B virus and the possibility of antitumor application of CRISPR-Cas9 by targeted mutation of the HBsAg gene leading to suppression of tumor progression of hepatocellular carcinoma have been demonstrated [38, 39].

Human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome, which remains one of the most serious health care problems worldwide [40]. According to [41] CRISPR-Cas9 method can prevent the development of HIV-1 infection. Other studies have shown the use of CRISPR-Cas9 method for the treatment of HIV infection [37, 42, 43].

Agriculture and food technology

The emergence of agriculture has been the basis for civilization. In human history, the availability and accessibility of food has been critical and enormous efforts have been expended to obtain it. Today, due to growth of global population, access to food is becoming more problematic [44]. New stress tolerant and efficient crops are needed. This can be achieved by CRISPR-Cas9 technology [45, 46].

Plant-specific RNA polymerase III promoters are used for the successful operation of Cas9 protein and guide RNA (gRNA) in plant cells.

These promoters are called tU6 (Arabidopsis), TaU6 (wheat), OsU6 or OsU3 (rice).

There are several commercial vectors for the expression of these Cas9 or Cas9 and gRNA variants in plants. Addgene, a global collection of plasmids provides over 30 "blank" gRNAs for binary vectors. These gRNAs include a plant RNA polymerase III promoter, gRNA as a vector where the desired gRNA can be inserted [47].

Tomato cells are often used for CRISPR-Cas-mediated modifications. Leaf shape studies have shown that genetic mutations created by CRISPR-Cas technology are inherited. The gene SlAGO7 (Argonaute7), gives a flat appearance to tomato leaves, but deletion of this gene using CRISPR-Cas9 technology results in needle or wire-shaped leaves [48]. Genome editing was carried out to create cocoa varieties that are resistant to pests, higher yielding, drought tolerant, with improved flavor and seed quality. For this purpose, the TcNPR3 gene was deleted using CRISPR-Cas9 technology [49].

Successful studies have been conducted on rice and wheat, important food sources. In the case of rice, approximately 92 % of the studies are based on CRISPR-Cas9 technology.

Abscisic acid receptors affect rice yield [50]. CRISPR-Cas technology allowed simultaneous mutation of genes encoding abscisic acid receptors (PYL1), PYL4 and PYL6, resulting in a marked increase in growth and yield in rice [51]. Mutations in wheat genes such as PDS, MLO and NAC2 have been investigated. Targeted mutations in MLO gene obtained using CRISPR-Cas9 technology resulted in plants resistant to yellow rust disease [52, 53].

CRISPR-Cas9 technology can be used to accurately and efficiently reduce α-gliadin content to reduce immunoreactivity of durum wheat products for consumers with gluten intolerance [54]. Direct gene editing was shown [55] in cotton plants using CRISPR-Cas9. In a study [56], plants modified with CRISPR-Cas9 showed resistance to cucumber mosaic virus and papaya ringspot virus infection.

CRISPR-Cas can be used to produce efficient animal feed. Since 2017, 36 varieties of soybean and corn have been approved and authorized for use as animal feed [57].

Harmful microorganisms can cause food poisoning and food spoilage, while beneficial microorganisms help preserve food and promote a healthy digestive system [58]. In food biotechnology, CRISPR systems are used in antiviral vaccination of bacterial cultures, genotyping, antibiotic resistance monitoring, and modification of probiotic cultures [59, 60].

The CRISPR-Cas9 system was first applied by Danisco in 2008. The company used it to enhance the immunity of bacterial cultures to viruses, and nowadays many food manufacturers use this technology to produce cheese and yogurt. Probiotics are known as live microorganisms that benefit the host when consumed in sufficient quantities [61]. The successful antiviral vaccination of Streptococcus thermophilus starter culture used in fermentation of dairy products has promoted the use of CRISPR-Cas in the food industry [62]. CRISPR-Cas system is applicable for pathogen control, food safety, and shelf life extension [63]. A study [64] used CRISPR loci for genotyping to distinguish strains in products with mixed microbiota, especially those produced by fermentation. One early study was conducted on Lactobacillus buchneri, which causes spoilage in pickled foods, especially cucumbers, by altering the flavor. After identifying the formation and diversity of CRISPR-Cas systems in L. buchneri, the use of a 36-nucleotide CRISPR type 2-A locus for identification yielded successful results [65]. CRISPR loci has been used for genotyping in Enterococcus faecalis, which is used in fermentation of meat products, as well as in Lactobacillus gasseri and Bifidobacterium, known as probiotics [66].

The CRISPR-Cas9 system of the second type was used on E. coli and Staphylococcus aureus bacteria to test its antibacterial potential [67]. It was shown that the CRISPR-Cas system can be used to inhibit and kill antibiotic-resistant bacteria. An effective method to remove carbapenem-resistant plasmids and restore sensitivity in bacteria to antibiotics using the CRISPR-Cas system has been shown. CRISPR precisely targets and destroys antibiotic-resistant bacteria, facilitating the identification of resistance mechanisms, which opens new possibilities in diagnosis and therapy [68].

Genome editing techniques have been used in animal breeding to improve disease resistance, improve product quality and efficiency, and produce drugs for biomedical purposes [44, 69].

In 2018, a successful editing of the MSTN KO gene in goats was carried out. Using CRISPR-Cas9, the third exon of the gene was altered, resulting in a significant increase in average daily weight gain compared to the control group. The offspring obtained from the edited animals retained the altered genotype and corresponding phenotype, as well as high genetic stability and fertility [70]. Thus, the CRISPR-Cas9 system is a tool for creating new lines and breeds of animals with economically useful traits.

In a study on the commercial production of human interferon in transgenic chickens [71], the CRISPR-Cas9 system was used to insert the human interferon beta (hIFN-b) gene into the chicken ovalbumin gene, resulting in the biologically active hIFN-b protein appearing as part of the egg white.

Using the CRISPR-Cas9 system, the myostatin protein gene was successfully blocked [72], resulting in a significant increase in muscle mass in animals of the breed under study.

CRISPR-Cas9 technology is applicable together with breeding methods aimed at increasing animal productivity [73, 74], and at increasing resistance to infectious or non-infectious diseases [75, 76], and also helps to control the desired sex in farm animals [77].

In a study conducted on goats [78], it was shown that blocking the fibroblast growth factor 5 gene resulted in an increase in fiber length in cashmere goats. Genetically edited animals with blocked FGF5 gene were obtained [79].

Three economically important characteristics including fiber diameter and length showed that CRISPR-Cas9-edited goats with blocked FGF5 gene have increased total productivity.

CRISPR-Cas9 technology [80] allowed activation of AANAT and ASMT genes responsible for melatonin synthesis in sheep mammary gland epithelial cells. The melatonin content increased in the milk of ewes with activated genes compared to the initial ones.

Among recent advances, we should mention Genus, which used CRISPR technology to obtain a line of pigs fully resistant to porcine reproductive and respiratory syndrome virus [81].

Diagnosis of infections

CRISPR-Cas systems are used in a variety of analytical methods for DNA detection [82, 83], including SARS-CoV-2 [84], and the CRISPR-Cas13 system, which recognizes RNA, is used to diagnose infections caused by RNA-containing viruses [85]. CRISPR-Cas-based diagnostic systems have several advantages: high specificity, high sensitivity, simplicity and low cost. Diagnostics for the following pathogens have been created: on the basis of Cas9 protein — Zika virus, on the basis of Cas12 protein — HIV-1, hepatitis B virus, human papillomavirus, tuberculosis, SARS-CoV-2, on the basis of Cas13 — SARS-CoV-2 viruses, dengue fever and Zika virus, the diagnostic procedure is 3-4 hours [86].

It is amazing that after the discovery of CRISPR technology, the cost of genetic engineering decreased by 70–80 %. There is no doubt that CRISPR has great potential to change the diagnosis and therapy of human diseases, biotechnology of viruses, plants and animals, and biotechnology in general [37, 44].

In conclusion, despite significant public support for CRISPR-Cas technology, ethical and safety concerns remain and it remains one of the most debated applications.

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CRISPR-Cas әдісімен өңделген генді биотехнологияда қолдану

CRISPR-Cas жүйесі — ДНҚ нуклеотидтер тізбегі мен ген функцияларын дәл және мақсатты өзгертуге мүмкіндік беретін қуатты геномды өңдеу құралы. Оның қазіргі биотехнологияда көптеген қолданбалары бар, соның ішінде генетикалық ақауларды түзету, ауруларды емдеу және алдын алу, жануарлардың тұқымдарын жақсарту және ауылшаруашылық дақылдардың өсуі мен төзімділігін арттыру. Ұсынылған шолуда CRISPR технологиясының бактерияларды вирусқақарсы қорғаудың табиғи механизмінен генетикалық инженерияның соңғы әдісіне дейін қалай дамығанын қарастырылған. Бастапқыда бактерияларды вирустық шабуылдардан қорғану үшін арнайы РНҚ мен Cas ақуыздарын қолданатыны анықталды. CRISPR жүйесінің құрамдас бөліктерін гендерді басқару үшін басқа организмдердің жасушаларына тасымалдауға болатыны белгілі болды, яғни бұл процесс «генді түзету» деп аталады. Геномды түзету кезінде генетикалық нұсқаулар өзгереді, нәтижесінде кодталған ақуыздар мен жасушалық процестердің белсенділігі өзгереді. ДНҚ-ның кодтау бөлігіне және ДНҚ-ны қалпына келтіру механизмдеріне кесінділерді енгізу арқылы мақсатты өзгерістер үшін қажетті ДНҚ фрагменттерін енгізуге болады. CRISPR технологиясы муковисцидоз, орақ жасушалы анемия, суқараңғы сияқты және т.б. адамның генетикалық ақауларын тиімді түзете алады. Бұл зерттеулер адамдарға терапевтік қолдануға жол ашты. CRISPR қатерлі ісік пен тұқым қуалайтын ауруларды емдеуге және соқырлықты тудыратын, Лайма ауруының алдын алуға және безгектің вирустық векторлардан адамдарға берілуіне сыналды, сонымен қатар жануарлар үлгілерінде адамның иммун тапшылығы вирусынан жұқтырған жасушаларды жою әдісі сыналды. Шолуда биотехнологияның негізгі технологияларының бірі ретінде әртүрлі организмдер геномдарының мақсатты учаскелеріне CRISPR өңдеуді қолданудың ең маңызды мысалдары келтірілген.

Кілт сөздер: CRISPR, Cas, геномдарды өңдеу, биотехнология.

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Редактирование генов методом CRISPR–Cas — применение в биотехнологии

Система CRISPR-Cas — это мощный инструмент для редактирования геномов, позволяющий точно и направленно изменять последовательности нуклеотидов ДНК и функции генов. Он имеет множество применений в современной биотехнологии, включая исправление генетических дефектов, лечение и профилактику болезней, а также улучшение пород животных и увеличение роста и устойчивости сельскохозяйственных культур. В предлагаемом обзоре рассматривается, как технология CRISPR развивалась от природного механизма противовирусной защиты бактерий, до новейшего метода генетической инженерии. Первоначально было выявлено, что бактерии используют специальные РНК и белки Cas для защиты от вирусных атак. Оказалось, что компоненты системы CRISPR можно перенести в клетки других организмов для управления генами в процессе, называемом «редактированием генов». При редактировании генома происходит изменение генетических инструкций, в результате чего изменяется активность кодируемых белков и клеточных процессов. Вставляя разрезы в кодирующую часть ДНК и используя механизмы восстановления ДНК, можно вставлять желаемые фрагменты ДНК для целенаправленных изменений. Технология CRISPR может эффективно исправлять генетические дефекты человека, такими как муковисцидоз, серповидно — клеточная анемия, катаракта и др. Эти исследования открыли путь к терапевтическому применению на людях. CRISPR был протестирован для лечения рака и наследственных заболеваний и вызывающих слепоту, предотвращения болезни Лайма и передачи малярии от вирусных переносчиков к людям. Также на животных моделях был проверен способ избавления инфицированных клеток от вируса иммунодефицита человека. В обзоре приводятся наиболее значимые примеры применения редактирования CRISPR целевых участков геномов различных организмов как одной из ключевых технологий биотехнологии.

Ключевые слова: CRISPR, Cas, редактирование геномов, биотехнология.

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