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Using the CRISPR-Cas9 System in Genetic Engineering Applications : A review Article

Dr. Abdalkader Saeed Latif[1] , Ammar Basim Al-Asadi[2], Dr. Mahmood Abed Hamzah[3]
Dr. Reyam Naji Ajmi [4]

1. National University of Science and Technology / College of Health and Medical Technology
2. Collage of Ibn Sina University of Medical and Pharmaceutical Sciences, Iraq, Baghdad
3. Iraqi Ministry of Education, Iraq, Baghdad
4. Department of Biology Science, Mustansiriyah University, POX 46079, Iraq-Baghdad.

abdalkaderlatif@yahoo.com, ammar.alasadi@ibnsina.edu.iq , mhmodalqaus@yahoo.com

Abstract : It has been described the CRISPR-Cas9 system ,the most exciting achievement in biomedical research since the dawn of genetic engineering in the 1970s, with the broad scope to better understand and treat human and animal diseases and its potential to revolutionize medical and agricultural research by identifying the double helix of DNA and the role it in the genetic formation of living organisms. The CRISPR tool is a giant step forward in this regard, and compared to existing research tools, it is a fast, easy, reliable, and relatively low-cost way to target and refine specific genetic sequences. Therefore, this paper discussed the importance of this technology, the way genes work in cells, and the most effective new medical treatments for a group disease that destroys the living body.

Keywords: CRISPR-Cas9 technology, Genetic Engineering, Applications

A review Article Problem: More research is needed to evaluate whether using CRISPR-Cas9, a type of molecular "scissors" used to edit genes, activates a mechanism designed to protect cells from DNA damage.

A review Article Objective: Scientists are attempting to use CRISPR, a genetic modification technology that permits the change of the DNA of living things, including people, to treat diseases, despite concerns about it.

The method of the article: The CRISPR system's mode of action demonstrates that any sequence of guide RNA may be synthesized on the Cas9 enzyme, since the enzyme can be used to look for any DNA, not just DNA found in viruses that have the same sequence as the gRNA it carries. As a result, every living thing's genome can now be altered by modifying its genetic DNA in a way that is more adaptable and simple than with previous methods of genetic modification , the two components of this cutting system the gRNA and the cas9 cutting protein are collectively referred to as CRISPR technology.

1-Introduction

The history of CRISPR areas was initially uncovered in 1987 by Japanese researchers at Osaka University. While researching particular genes in the bacteria E. Coli, they unintentionally

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sequenced a section of DNA that was close to the gene under study and included a lot of repeats of similar sequences. The commas that separate them have an unknown function, although in 2005, it was proposed that CRISPR is a component of the bacterial immune system. When researching the lactic acid-producing *Streptococcus thermophilus* bacteria and their defense mechanisms against viral infections in 2007, which were one of the main issues facing the dairy and cheese industries, scientists discovered that CRISPR is an adaptive immunity mechanism used by bacteria when exposed to viral attacks [1].

In 2011, following a thorough investigation into the workings of the CRISPR system, researchers found that any guide RNA chain could be synthesized onto the Cas9 enzyme. The enzyme could be used to look for any DNA with the same gRNA sequence, not just viruses, when they did, which was in 2013. As a result, scientists can now modify the genome of any living thing by cutting its genetic DNA in a way that is more flexible and straightforward than previous genetic modification techniques. This cutting system, which is typically referred to as CRISPR and is made up of two components: the gRNA and the cas9 cutting protein, has become the most popular method of genome modification [2].

1-1 CRISPR as a defense mechanism:

It has been shown that prokaryotes, like bacteria, use CRISPR sequences as an adaptive immune system to defend themselves against foreign genetic elements (such phages and plasmids) that they come into contact with. When a bacteriophage virus, for instance, infects a bacterium, it injects its DNA into the bacterium, causing it to multiply within the bacterium to the point where it could be fatal. The bacteria then release enzymes to gather fragments of the virus's DNA, which they then store in CRISPR by insertions and deletions of spacer DNA that has been found to match portions of the virus's genetic code. Therefore, the bacteria copy the DNA contained in CRISPR into RNA, known as crRNA, when they are exposed to a similar virus again. The cas9 truncation protein, which cas9 employs to identify the virus, is connected to the crRNA. The cas9 enzyme appears to ride the two strands of the virus's DNA when it comes into contact with it, searching for a match between the virus's DNA and a portion of the crRNA it contains. This segment of the crRNA, which is about 20 nucleotides long, is what is used to identify the virus. The two strands of DNA split if the 20 nucleotides carried by cas9 match the 20 nucleotides of the virus's DNA. This prompts cas9 to cut the two strands of DNA, destroying the virus [3].

1-2 CRISPR region assembly:

The CRISPR region consists of regularly spaced, palindrome repeats of nucleotide sequences, between which are small, unique pieces of spacer DNA, It is worth noting that there are several types of CRISPR systems that differ depending on the type of bacteria, so the composition of the CRISPR sequence, its enzymes, and the method of transcribing crRNA can differ from one type to another. The CRISPR region includes a promoter region to ensure that the CRISPR region can be read and translated into CRISPR-RNA or crRNA that is used by CRISPR-related enzymes to recognize the target, the stimulus is followed by short repeats with unique spacers [4]:

Palindrome symmetry in the DNA strand results in an active shape stem-loop in RNA :

A- Palindromic symmetry , B: Loop , C: Stem

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As for repeats, they are already present in bacterial DNA, and are copied like the constant part of crRNA. They are longer parts, usually 28 to 37 base pairs in length, but can be shorter or longer than that. Some CRISPR repeats are in the form of palindromic sequences, meaning that each strand of DNA contains a sequence of nucleotides such that if that sequence is read from the 5' to 3' end of one of the two strands, it will be completely identical if it is read from the end. 5' to 3' in the connecting strand, which together form the DNA double helix. This symmetrical sequence means that it copies base-bonded RNA, thus forming a folded RNA strand with a stable, effective shape, in the form of a stem-loop that helps cas enzymes recognize it, while some CRISPR repeats are structured so that the resulting RNA is not folded into a specific shape [5]. Spacers are the parts that have been added from the attacking viral DNA, and are copied as the variable part of the crRNA that connects to the enzyme cas9, which is used to recognize matching DNA. Its length, like DNA, is usually between 32 and 38 base pairs (but ranges from 21 to 72 base pairs). New breaks may appear within a short period as part of the immune response to the viral attack on the bacteria [6]. A single CRISPR area typically consists of fewer than 50 spacer repeat sequences. CRISPR-related genes, which are genes next to CRISPR, provide the genetic code required to make CRISPR-related proteins, such as the Cas9 enzyme. The area that builds the RNA molecule known as tracrRNA comes after the CRISPR sequence and spacers directs the cutting molecules and crRNA to their intended positions on the DNA [7].

That is, in nature, the total RNA that connects to the cas9 enzyme consists of these two parts: the fixed part that does not change with the change of the target, according to some sources is called tracrRNA (and others consider it part of crRNA) and it has another role in bacteria in forming the variable part that is similar to acid. The nuclear virus, which is called crRNA, is used by the enzyme to recognize the target and consists of approximately 20 nucleotides. They usually form the fixed part and the variable part together, guide RNA, or gRNA for short, and this name (gRNA) is usually given to synthetic RNA, not bacterial, which is only called crRNA[8].

1-3 CRISPR-Cas technology in genetic modification:

The two DNA strands open up to reveal the cutting area matching to the gRNA, which attaches to one of the strands to cause the cas9 enzyme to cut the two DNA strands. Scientists have used the CRISPR system, which is already present in bacteria, to precisely cut a specific section of an organism's genetic DNA in order to remove genes that cause the organism to exhibit an undesirable trait or replace those genes with other genes. This allows scientists to modify the genome of any type of organism [3].

The CRISPR-Cas9 system consists of two main components: the guide RNA molecule, also known as gRNA for short, serves as a guide to direct the cas9 enzyme to a specific sequence of DNA to make a cut at that region of variable portion of gRNA, which consists of approximately 20 nucleotides. The Cas9 protein, also known as CRISPR associated protein 9, is a non-specialized endonuclease that cuts two strands of DNA like molecular scissors, and it goes to the region to be cut based on the gRNA it carries and other factors like the availability of a special recognition sequence in the DNA called the PAM sequence [9]. The use of a gene-editing tool called CRISPR, scientists may alter DNA sequences and alter the way that genes work. This technology can be used to heal diseases, improve crop growth, and fix genetic faults, among other things, despite being a promising technology, there are a number of ethical questions it brings up. CRISPR-Cas9 is referred to by the acronym CRISPR.

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DNA stretches with the protein Cas9, an enzyme that functions as molecular scissors and can cut DNA strands, make up CRISPR. [10].

CRISPR technology originated from the natural defense systems of single-celled organelles known as microorganelles, such as bacteria and archaea. These organelles defend against viral invasion by utilizing several Cas proteins and RNA produced from CRISPR DNA. In order to use the viral DNA as a weapon against future invaders, the organelle chops it up and organizes segments of it within its genome. The CRISPR components have the ability to modify genes through genetic alteration when they are transplanted to a more complex organelle. The primary building blocks of CRISPR are pairs of nucleotides, either an adenine to a thymine or a cytosine to a guanine, which make up a double-stranded DNA molecule [4].

The term CRISPR refers to clustered regularly interspaced short palindrome repeats, which are repeats of a sequence of repeats in DNA with spacers inserted in between each repeat. When we talk about repeats in the genetic code, we're talking about the base pair order on the DNA molecule's helical ladder. These bases repeat in the same order throughout the CRISPR segment of DNA, and these repeated sections result in symmetrical sequences known as palindromic sequences. This means that, in certain cases, such as the word "racecar," which is read in both directions, the bases on one strand of DNA match those on the other strand when read in reverse short palindrome repeats are evident throughout all CRISPR regions of the DNA and spacers are placed between each repetition when bacteria attack viruses, they take these spacers from the viruses and integrate a portion of the viral DNA into their own genome by serving as a kind of memory bank, these spacers help bacteria identify viruses in the event that they assault them again [11]. In a 2007 publication, a group of Danisco researchers also detailed this procedure. Using the dairy product-found model bacterium Streptococcus thermophilus, they saw that following a viral infection, the bacteria added additional spacers to their CRISPR regions, these spacers' nucleotide sequences are exactly the same as certain regions of the virus' genome [9].

CRISPR regions function as a kind of viral memory bank in DNA; but, in order for the information they store to be utilized in other parts of the cell, they need to be copied into RNA, another type of genetic material. CRISPR RNA can migrate throughout the cell and work with proteins to build molecular scissors that cut viruses into tiny pieces, in contrast to DNA sequences, which stay inside the DNA molecule. One strand, as opposed to two, is the difference between DNA and RNA. That is, it appears to be only partially a ladder. A segment of CRISPR in the DNA serves as an anchor for the creation of an RNA molecule, and polymerase proteins produce an RNA molecule complementary to that strand, meaning the bases of the two strands will fit together like puzzle pieces. For instance, the transcription of the G in DNA into the C in RNA occurs. Every CRISPR RNA snippet comprises a duplicate of a terminator and repeat from a CRISPR DNA region. To protect bacteria against viruses, CRISPR RNA collaborates with the Cas9 protein and another RNA type known as tracrRNA [12]. Case 9. This enzyme breaks down DNA from other sources, the protein will be cut when it attaches to CRISPR and tracrRNA. DNA to be cut is complementary to a 20-nucleotide length of CRISPR RNA. Both strands of helical DNA are cut by the Cas9 enzyme protected from making any cuts in the genome by a safety mechanism. PAMs, or pseudorandom amino acid sequences, function as signals next to the target DNA region. One explanation for why Cas9 may not attack the bacteria's DNA is that it will not cut the target sequence if the Cas9 enzyme complex does not see PAMs close to it [11] .

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1-4 How does CRISPR work as a gene editing tool

DNA sequences contain strings of instructions that are encoded in them. To modify the genome, one must alter these sequences and the messages they carry. This can be accomplished by making cuts in the DNA and deceiving the cell's defense mechanisms into making the desired changes. One method for doing it is CRISPR-Cas9. The natural CRISPR system could become an easy-to-use, programmable tool for genome editing if bacterial CRISPR-Cas9 is able to cut any DNA, not just viral DNA. Scientists may easily alter the CRISPR RNA sequence that binds to the complementary sequence in the target DNA to instruct Cas9 to cut a specific section of DNA. In a 2012 study, scientists created "directed RNA" by fusing CRISPR with tracrRNA, which simplified the method. Therefore, a guide RNA and the Cas9 protein are all that are needed for genome editing [12]. This makes it possible to determine the complementary RNA's sequence. Church stressed how crucial it is to confirm that the selected nucleotide sequence is exclusive to the target gene and not found anywhere else in the genome. "The DNA is then only cut at this precise location by the RNA and Cas9 protein, acting like scissors. The cell's natural repair processes take over and attempt to put the broken DNA back together. Modifications to the genome are now possible. According to, there are two methods for doing this. [13] :

First, reassemble the two sections using adhesive. When using this technique, called non-homologous end joining, mistakes can be made by inadvertently adding or removing nucleotides, which might result in mutations that render the gene inoperable.

Second, Subsequently, a nucleotide sequence is added to the break to seal it, abrief DNA strand serves as the cell's template. Researchers can select a DNA template to transcribe any desired gene or to reverse a mutation.

1-5 Who discovered CRISPR technology:

In 1987, scientists from Osaka University in Japan made the discovery of CRISPR in Escherichia coli bacterium. The biological relevance of these DNA sequences was first unknown to scientists, and the term had not yet been coined. After genetic analysis methods advanced in the 1990s, numerous other researchers discovered CRISPR sequences in various other microbes. Francisco Mojica, a scientist at the University of Alicante in Spain, was the first to characterize the unique traits of CRISPR and also came up with the name, which was first used in a general article. 2002, prepared by Ruud Jensen of Utrecht University and published in the Journal of Molecular Microbiology [14].

The 2020 Nobel Prize in Chemistry was shared by scientists Emmanuelle Charpentier, director of the Max Planck Unit for Pathogen Science, and Jennifer Doudna, professor of biochemistry, biophysics, and structural biology at UC Berkeley. They are credited with developing a gene-editing tool system using the bacterial CRISPR/Cas system. According to biochemist Feng Zhang of the Broad Institute, mammalian cells are where the CRISPR system functions. The Broad Institute was granted the first patent for modifying eukaryotic genes using CRISPR technology as a result of this finding [13,23].

1-6 How is CRISPR used

According to [2,3,10] Further research in lab settings and on animal models of human diseases has demonstrated the efficacy of this method in repairing genetic abnormalities. Studies on conditions like cystic fibrosis, cataracts, and Fanconi anemia cleared the path for their therapeutic use in people. Early clinical trials have investigated the use of CRISPR in treating blindness-causing genetic disorders and cancer. It investigated a method to stop malaria and Lyme disease from

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spreading from viral vectors to people. Neville Sanjana, an assistant professor of biology, neuroscience, and physiology at New York University and director of the New York Genome Center, stated, the public perception of CRISPR is very focused on the idea of using gene editing clinically to treat diseasewhile there are many more applications for technology, this is undoubtedly a fantastic usage of it. Additionally, CRISPR has been used in the food and agriculture sectors to cultivate beneficial microbial cultures and safeguard these bacteria against viruses in processed foods like yoghurt. It is applied to crops to increase yield, increase their resistance to drought, and enhance their nutritional qualities [10]. Gene drives, which are genetic engineering techniques that increase the likelihood of a particular feature being passed down from parents to offspring over the course of generations and the trait spreading to large groups of this sort, are one potential application. Gene drives can be employed for a number of purposes, including eradicating exotic crop species and reversing crop herbicide and pesticide resistance. The CRISPR-Cas9 technique has been utilized to create a number of viral infection diagnostic assays during the COVID-19 pandemic [14].

CRISPR technology has recently been used in the following ways according [10,11]:

On August 2, 2017, researchers reported in the journal Nature that they had utilized CRISPR to successfully correct an embryonic abnormality that was causing heart disease, according to research published in the journal BioNews, on April 16, 2018, researchers developed CRISPR technology to edit thousands of genes simultaneously. However, despite its many applications, CRISPR technology has some disadvantages, the main one is that it is not 100% effective, as noted by Church. That is, just a portion of the target DNA may be modified by CRISPR technology. Since DNA is cut in places other than the intended target, the procedure may also have unexpected effects, there could be target mutations or incorrect modification a phenomenon known as genome vandalism[12].

1-7 Potential risks and ethical concerns of using CRISPR technology

There are concerns regarding the advantages of CRISPR and the moral implications of genome editing given its wide range of prospective uses. A number of ethical concerns were brought up in particular in 2018 when He Jiankui, a biophysicist who was previously employed at Shenzhen's Southern University of Science and Technology, revealed that his team had edited human embryos' DNA—the first instance of gene editing involving human beings—and was later given a prison sentence. The three-year fine of $560,000 is for practicing medicine without a license, forging ethical review paperwork, and breaking Chinese regulations regarding reproductive procedures, this research prompted concerns regarding the regulation of CRISPR use [14]. The term "germ line editing" refers to the process of making genetic alterations to human embryos and reproductive cells, such as sperm and eggs. Since changes made to these cells have the potential to be handed down to future generations, there are a number of ethical issues with utilizing CRISPR to create adjustments in this area. 2014 revealed the possible effects of gene drives on the environment: Through hybridization, traits added to one group may multiply to include other creatures. The National Academies of Sciences, Engineering, and Medicine have developed a thorough report and recommendations regarding genome editing in the event that the use of sex line modification evolved from a therapeutic tool for enhancing human characteristics. Gene drives can also reduce the genetic diversity of the target population to make changes that might affect future generations without their consent [15, 24].

The National Academies stresses that "caution does not mean prohibition" and advises against attempting sex line alteration. Only when there are no other viable therapy options for genes that

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cause significant disorders do they advise germ line modification. The trial organizers follow up with the participants' relatives several generations after the experiment ends to observe the ongoing changes in the genome over time. This is done in order to comply with the requirement to gather information on health risks and benefits and to maintain ongoing supervision during clinical trials [16].

1-8 Genome screening systems

Human intervention in altering creation, the scientists killed the cells they had altered rather than planting them in the wombs of women in order to increase thThis was the approach taken by scientists at the Francis Crick Institute in London in their quest t o investigate and comprehend the early stages of embryonic development. With their capacity to produce precise and targeted modifications, effector-like nucleases (TALENs) and zinc finger nucleases (ZFNs) have greatly advanced genome editing technologies over previous methods , two proteins, one of which identifies certain nucleotide sequences and the other is the core of ZFNs and TALENs. The insertion of some mutations can be facilitated by nucleases' ability to cleave double-stranded DNA upon detection. It is necessary to create a unique nucleotide sequence-recognizing protein for every experiment in order to alter the genome. The creation of target molecules for every knockout or knockin experiment is challenging and time-consuming due to the requirement that nucleoprotein-acid interactions guide editing [15,17].

The discovery and subsequent development of the CRISPR-Cas9 system, a genome editor that allows targeted cleavage of a sequence of interest by an endonuclease that directs an RNA molecule, has been the most significant achievement in the field of genome editing. This was a substantial advancement over prior genome editors, which depended on protein-nucleic acid interactions. Because the CRISPR-Cas9 method requires an RNA molecule to target the targeted region, each experiment can be created using typical base pairing criteria [18].

The three essential components of the CRISPR-Cas9 system are the Cas9 enzyme, single guide RNA (sgRNA), and donor DNA (DDNA). This is how the system is put together. SgRNA is made up of the scaffold, a lengthier tract of SgRNA, and the protosperm, a 20-nucleotide fragment. Because the protoplast area directs the editing system to the desired location, it must be redesigned for each experiment. Regardless of the region to be targeted, the scaffold is the same RNA sequence that physically binds with the Cas9 enzyme to generate a ribonucleoprotein (RNP). The Cas9 enzyme facilitates lifting the target DNA by employing the protospacer as a guide to locate this place. Its applicability is determined by the study. DDNA is thus suitable for gene knockin and gene knockout research, which require inserting a marker or antibiotic resistance gene into the genome in place of the desired gene. This is because DDNA carries the sequence required by the Cas9 enzyme to accurately insert into the area it is cutting. Furthermore, new sequences can be added to the genome by synthesizing DDNA. When gene truncation is required, for example, an in-frame stop codon can be placed into a specific location of the target gene, as outlined below. Other uses include the insertion of tag sequences or the insertion or modification of tag sequences in certain gene areas, such as a functional domain [23].

The adaptability of CRISPR-Cas9 technology is one of its key advantages. This versatility is demonstrated by the fact that the Cas9 enzyme can be introduced into the host cell in one of three

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forms—RNA, protein, or both—depending on the transduction pathway used. The cas9 gene is frequently put on the plasmid, along with a selection marker and a cassette for sgRNA expression. The fundamental benefit of this approach is that it only requires one construct to successfully transform into a cell, ensuring the presence of all the elements required for CRISRP-Cas9-mediated genome editing. However, this strategy is dependent on the availability of the host species' expression system. Cas9 cannot effectively cause damage unless it is expressed at high quantities, which requires a suitable and maybe specialized promoter may be a disadvantage for non-model species in which these promoters have not yet been developed; in these instances, it may be more convenient to insert Cas9 into the RNA or protein form. When introducing RNA into a cell, there are extra challenges because the material is unstable and may not survive the transformation process. Furthermore, whether the Cas9 gene is provided as DNA or RNA, the host system may require modifications to the gene sequence [17] A cas9 gene optimized for usage in a mammalian cell, for example, may not work in a plant cell, and the Streptococcus cas9 gene may not work in a mammalian host cell. To circumvent these obstacles, the protein version of Cas9, which can be synthesized into an RNP and transfected into the host cell with the help of sgRNA, can be employed. Since this technique doesn't rely on self-expression or codon optimization, it ought to operate well in most non-model species. The protein-based strategy has the disadvantage of being incompatible with DNA-based transformation technologies such as agrobacterium-mediated transfer Therefore, in order for a protein-based approach to work, a transformation protocol such as those based on biological or plastic processes must be given [20] . This RNP-based system has been used successfully in filamentous fungi, *Fusarium oxysporum and Mucor circinelloides*,, There are currently no developed techniques for genome editing or transformation, research on *E. omanensis* has so far concentrated on the genetic factors of its unusual sexual cycle. Sexual reproduction in this fungus occurs only between isolates of the MAT1-1 and MAT1-2 mating types31. In contrast to MAT1-131, isolates of MAT1-2 from the closely related species *Huntella moniliformis* may reproduce sexually on their own and complete the sexual cycle even in the absence of a mate. MAT1-2-7, the mating gene, is assumed to be notably different in *H. omanensis*, where the transcript is full and complete, whereas in the gene is severely truncated. This variation in sexual abilities could be due to a shift in mating gene expression [18]. The MAT1-2-7 gene was shortened from *H. omanensis* to resemble the truncation observed to better describe the function of this gene in sexual reproduction [21].

1-8 Applications

Though genetically changing entire human cells is still a challenging task, scientists have so far been able to readily modify stem cells, red blood cells, or cells cultured in laboratory dishes. Researchers are currently using CRISPR-Cas9 as a key tool in gene drive technology, which they are using to try and eradicate malaria by genetically modifying mosquitoes to carry a trait that is resistant to carrying the disease, which spreads completely in subsequent generations when mated with non-malarial mosquitoes. This technology is widely used in the field of medical research because it allows researchers to disrupt different genes to discover how these genes affect the course of complex diseases like Alzheimer's or cancer. resistant to carrying the disease, as opposed to Mendel's natural laws, which dictate that the average likelihood of inheriting the trait is 50% [20, 24]. This is achieved by making the probability of inheriting the disease resistance trait at 100% in the ensuing generations. Scientific institutes in Switzerland have reported that they have successfully treated a

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patient with thalassemia type B through the use of CRISPR in the treatment of genetic blood illnesses such sickle anemia . Following the successful completion of gene-editing procedures in China and the United States, British scientists used CRISPR-Cas9 to modify some genes in human embryos. Because this is regarded as human intervention in altering creation, the scientists killed the cells they had altered rather than planting them in the wombs of women in order to increase the approach taken by scientists at the Francis Crick Institute in London in their quest to investigat e and comprehend the early stages of embryonic development. Because this is regarded as human intervention in altering creation, the scientists killed the cells they had altered rather than planting them in the wombs of women in order to increase the success rate of artificial fertilization. Such study on people is a topic of much discussion amongst physicians, scientists, legislators, jurists, and intellectuals. Such research is not allowed in Germany [17].

In 2018, a Chinese scientist named He Jiankui claimed that he applied the technology to human embryo cells and implanted them in the mother's womb before the twin fetuses "Lulu" and "Nana" were born, where he modified their genetic code to make them resistant to the HIV virus that causes AIDS, by He used CRISPR technology to disable the gene that produces the protein receptor CCR5, which noted that people whose genetic structure lacks the functional form of this receptor tend to be unusually resistant to AIDS and HIV, which caused a great stir in scientific circles for ethical reasons[18,21].

The protocol below details the to reach a mechanism for the CRISPR system to work from the guide RNA chain to the cas9 enzyme, with the possibility of using the enzyme to search for any DNA that has the same sequence as the gRNA that it carries, by cutting its genetic DNA in a flexible and easier way than other genetic modification techniques, which consists of two parts, the cutting protein. cas9 and gRNA, to create it following the failure of plasmid-based CRISPR-Cas9 genome editing methods and homologous recombination-based gene substitution[24].

2- Conclusions and Recommendations

The capacity to study a broad range of biological processes in a variety of organisms has improved due to the growing availability of fully assembled and assembled genomes. This is true for both model species and non-model species, many of which may offer a more diverse understanding of biological processes. These kinds of data have their own applications, such as whole-genome and clone comparisons, transcriptase network identification, and gene discovery. However, despite the remarkable velocity at which genes are predicted, annotated, and putatively linked to many functional pathways, the functional characterization of these genes is still lacking and constrained by the molecular toolkits that are currently accessible for many species. This is especially true for non-model species too difficult to produce genetic data [23].

Fin atomic examination of mutant strains obtained through either knockout or knockin studies can partially characterize the roles of particular genes crucial to the biology of fungal species. These two systems rely solely on the accessibility of genetic engineering protocols, which should at the very least comprise a genomic editing system and a transformation system. In much different filamentous fungus, several distinct transformation methods have been developed.4. Physical systems, including those derived from electrophysiology and biology have been developed for *Trichomyrma harzianum and Aspergillus niger*, respectively. Systems have been developed that

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use chemicals such as calcium chloride or lithium acetate for *Neurospora crassa*. Finally, biological systems based on the use of the *agrobacterium tumefaciens* have been used for successful transformation of *Ceratocystis albifundus*.
Different transformation procedures are widely available, although genome editing systems are less common. When conducting traditional functional characterisation studies on filamentous fungus, cleavage knockout marker constructs—selectable markers surrounded by homology sections to the target gene or area in the genome—are frequently used3. Targeted DNA repair (HR), which creates homologous recombination between the knockout construct and the region of interest, is the foundation of the technique. The gene of interest is replaced with the preferred marker sequence as a result of this recombination event. Regretfully, even though this has worked well for many species including *Cercospora nicotianae, Aspergillus fumigatus and Grosmannia clavigera,* Since homozygous recombination rates vary greatly throughout fungal species, this procedure is ineffectual and occasionally unsuitable for certain species.

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Author contributions

Corresponding Author: Dr. Reyam Naji Ajmi/ Department of Biology Science, Mustansiriyah University, POX 46079, Iraq-Baghdad
Email: reyam80a@yahoo.com ; ORCID: https://orcid.org/0000-0003-2623-6671

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