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Review Article

Gene Editing Technique: Homologue Recombination, Antisense mRNA, RNAi, Site-Directed Mutagenesis, and CRISPR/Cas9

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Abstract

Since the 1970s, molecular scientists have been searching for methods to modify DNA via biological repair processes. Thus, they would be able to fix genetic mutations or introduce new functions into the genome. In order to accomplish this goal, genome editing techniques were created. It has become considerably easier, more accurate, and less costly to modify the genome because to the advent of new techniques. There has been a surge in interest in genome editing's potential uses since these breakthroughs, both for basic research and for improving human health through treating or preventing illness and disability. Scientists may now modify DNA to alter physical characteristics like eye color and the likelihood of contracting illness thanks to advances in genome editing technology. Various methods are used by scientists to do this. Every medical advancement has its own set of advantages, hazards, regulatory difficulties and ethical issues that must be taken into consideration while using it. Genome editing raises a number of important challenges, including how to balance possible advantages against the danger of unintended damage; how to manage the usage of these techniques; how to include cultural values into pertinent medical and economic considerations; as well as how to respect the unavoidable disparities, founded in country cultures, that will impact attitudes on whether as well as how to utilize these technologies. The purpose of this study is to discuss the most recent advances in gene editing, as well as the potential benefits and drawbacks that may result from its use.

Keywords: Gene Editing; Homologue; Antisense; mRNA; RNAi; Mutagenesis

Introduction

It is feasible to examine new sorts of topics and come up with new answers with the help of upgraded or new technologies. Molecular biology has long been a goal of science and health researchers and practitioners looking to better understand the underlying biology of everything from embryonic development to the immune and brain systems. The significance of genetics in illnesses including sickle-cell anemia, developmental disorders, as well as cystic fibrosis, as well as problems including deafness, low stature, and blindness, has made significant progress. There is a hereditary component to the formation of many of these illnesses and ailments. Complex interactions between genetics, environment, and other variables remain largely unexplained in the development of many diseases. Genetic sequences, on the other hand, are just a small portion of the picture. Genome regulation, including how and when genes are activated and deactivated, is being intensively studied. Tissue differentiation is influenced by expression of genes and epigenetic changes that might affect cancer as well as embryonic development.

Genome-editing tools have emerged in the past years that allow researchers to fast and cheaply modify the genomes of a wide variety of cell forms and species. Genome editing is currently made possible by many key technologies including Homologue Recombination, Antisense mRNA, RNAi, Site-Directed Mutagenesis, and CRISPR/Cas9. It is now possible to alter an organism's DNA using genome editing. Genetic material may be introduced, deleted, or changed at specific sites in the genome using these methods. Using genome editing to prevent and cure human illnesses is a major focus of research. Genome editing is now being employed in research facilities to study illness in cells as well as animal models [1].

Scientists are presently investigating the safety and efficacy of this method in humans. Clinical and research trials are looking at it for several ailments, particularly single-gene genetic diseases, hemophilia, as well as sickle cell disease (SCD) [2]. Even more complicated conditions including cancer, cardiovascular disease, and HIV infection might benefit from this therapy and preventative method.

Gene editing is the process of altering a live organism's DNA sequence in such a way that it becomes uniquely tailored to the individual [3]. There are certain enzymes, called nucleases, which are designed to address a particular DNA sequence and create breaks into the strands of DNA, making it possible to remove old DNA and insert new DNA. Long-standing debates concerning the ethical and societal ramifications of human genetic engineering have gained new urgency in light of recent advances in gene-editing techniques [4]. In the past, many doubts have been raised about the use of genetic engineering to heal human illness or to enhance qualities like attractiveness or intellect. Nevertheless, with the emergence of gene-editing technology, these concerns would be no longer speculative, and the explanations to them may have a significant influence on healthcare and humanity.

Homologue recombination

In homologous recombination, nucleotide sequences are swapped between two DNA molecules that are similar or identical [5]. In meiosis, chromosome pairs from the male and female parents align such that comparable DNA sequences may cross across, or transfer, from one chromosome to another. Genomic diversity among kids may be traced back to this exchange of DNA between parents. DNA repair, proliferation, meiotic chromosomal segregation, as well as telomere preservation are all aided by homologous recombination (HR) [6]. By using DNA helicases, homologous recombination is carefully controlled [7]. Cell-cycle halt, genomic instability, and cancer development may all be the result of homologous recombination dysfunction or dysregulation [8].

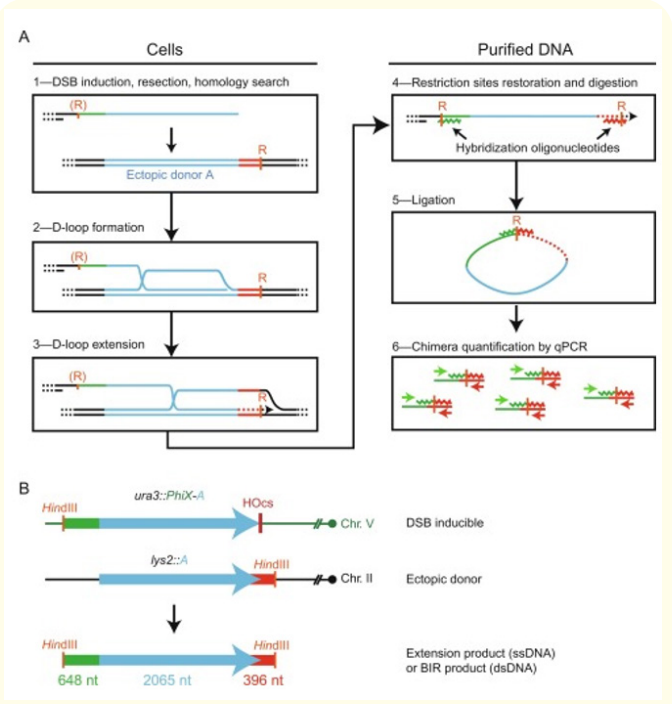

Figure 1: The Transfection and Repair Mechanism of Homologue Recombination Technique: Homology search and DNA strand invasion of an ectopically positioned donor are made possible by (1) site-specific DSB production and resection and (2) DNA strand invasion. (3) When the donor's 3′-OH end is primed for DNA synthesis, the donor's restriction site will be replicated downstream of the donor. The ssDNA molecule now has two restriction sites that are compatible with each other. (4) It is possible to digest the extended ssDNA after DNA extraction and reinstatement of restriction sites following annealing of long oligonucleotides. SsDNA with short DsDNA extremities is the result of this digestion. (5) A novel chimeric molecule is formed when these two extremities are ligated in close proximity under dilute circumstances. 6) Quantitative PCR is used to detect the quantity of this chimera in the cell population, and the readout is the number of D-loops that have been expanded beyond this restriction point on the donor. The dsDNA restriction site R and the ssDNA restriction site (R) are both designated by the letter R. (uncuttable). The ectopic donor, situated at LYS2 on chromosome (Chr.) II, was induced by site-specific DSB induction at URA3 on Chr. V in haploid S. cerevisiae. Because quick repair events might dilute the repair intermediates, the DSB can only be repaired by BIR [46].

An effective way for researchers to silence their desired gene was developed in the late 1980s [9]. An endogenous genetic material and a targeting vector were recombined in a homologous manner. The method's effectiveness was mostly attributable to two factors: ES cells were used instead of complete organisms in the Petri dish to identify the correct gene-targeting event [10]. When it came to making a final decision, there were two primary processes. Step one may identify those cells that have the incorporation of the targeting vector in their genome. Homology regions of the targeted vector were modified to include neomycin-resistant cassettes [11]. Step two of this procedure might detect ES cells in which the targeting vector was inserted into a random locus, rather than replacing an endogenous gene with the vector. Finally, thymidine-kinase cassettes, which are generally found in the vicinity of the homology area, were inserted into the constructs. Thus, the researcher was able to swiftly and effectively detect ES cells wherein the genetic material had been substituted by that of the targeting vector. There were no proteins produced by the targeting vector because it included a non-native sequence or a stop codon upstream of code sections, or both, and the interpreted protein was functionally so aberrant that it could not perform its intended biological function.

Homologous recombination (HR) permits the interchange of genetic material across and within species [12]. There is a growing body of evidence suggesting this mechanism plays an important role in microbial evolution, contributing to the homogeneity of microbial genomics and maintaining coherent population patterns. Maintaining the genome's integrity is a primary function of homologous recombination, which corrects many forms of DNA damage, including double-strand breaks [13]. An intramolecular continuous strand cannot be used to repair double-strand breaks. Homologous recombination, on the other hand, is a method that, via the reorganization of alleles gained by mutation, may impart genetic variety on a species' genome. This is thought to aid in the process of evolution. The ecological sustainability of organisms is taken into account while considering the function of genetic recombination. According to R. Fisher, "ecological stability" is a different concept from "fitness" in its original definition as the Maltusian parameter [14]. As a consequence of the mutations that occur in microevolutionary processes, the genetic transmission within the species restores a species-specific degree of ecological stability. As mutations that reduce the ecological stability of species accumulate, it is hypothesized that shortened selection will occur. Recombination's advantage in the A.S. Kondrashov model is explained by this form of selection (1982) [15]. In an evolutionary species, hybridization between narrow-specialized races results in an increase in ecological stability. For animals in evolutionary lineages, genetic recombination provides a continual DNA homogeneity and, hence, the species viability as a basic structure.

Genome stability may be maintained but can also be disrupted by the use of homologous recombination (HR). Even though homologous recombination is typically exact, redundant DNA in the genome and genes with varied copy numbers in the population is susceptible to occasional uneven crossing over during meiosis, resulting to variances in clinically significant features such as medication responsiveness, common illnesses such as thalassemia or autism, or anomalies of sexual differentiation [16]. In somatic cells, homologous recombination occurs less often but is nevertheless a normal and fundamental element of meiosis. One of the reasons for cancer's genomic instability is due to abnormalities in somatic recombination.

Antisense mRNA

The word "antisense" is used to denote one of two DNA strands, or in rare situations, RNA as well [17]. Due to the fact that RNA and DNA cannot be translated in both ways, DNA has two strands: one termed the sense strand and one known as the antisense strand. The coding side refers to the information on the sense strand that can be read by RNA. However, while there is production of RNA, the proteins that are engaged in the production of RNA read the antisense strand, which is non-coding, in order to provide sense for the mRNA [18]. Antisense RNA, a relatively recent discovery, is a second feature of antisense. Because they read in the opposite direction from how mRNAs do, these anti-codon-reading RNAs may either destroy or inhibit the expression of the mRNA codon that they attach to. It's a novel approach to gene control that's only been discovered lately.

In order to prevent protein translation, antisense RNA hybridizes with mRNAs that code for proteins and prevents them from being translated into proteins [19]. Both prokaryotes as well as eukaryotes have been shown to have asRNAs. AsRNA's major role is to control gene expression. In addition, asRNAs may be synthesized and used as research tools for gene suppression [20]. They might potentially be used to treat illnesses. Either transcriptional inhibition of the RNA complementary to the illness protein or post-

transcriptional gene silencing (PTGS) allow for the reduction of mRNA expression levels. AS ODNs post-transcriptional inhibition of mRNA expression may be replaced by transcriptional arrest, strand encroachment and triple-strand synthesis are two separate methods for halting transcription in double-stranded DNA.

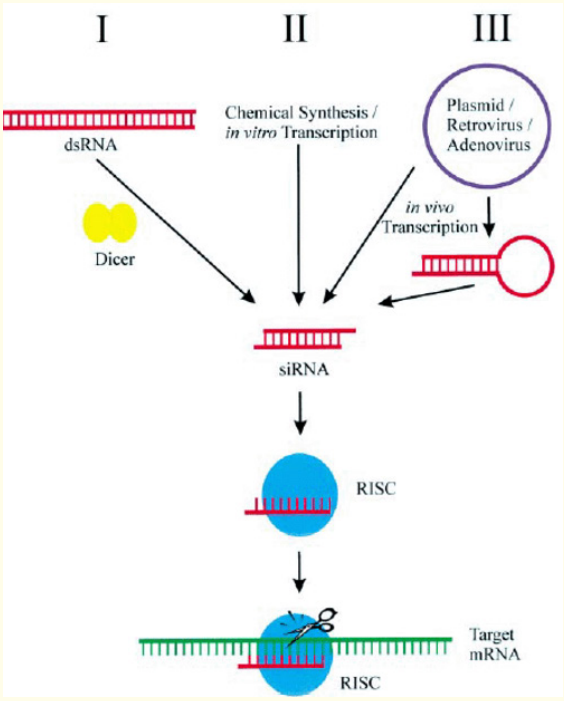

Figure 2: Transfection and Repair Mechanism of Antisense mRNA Technique: RNAi is activated by siRNAs, which may be synthesized in three different methods and then used to target certain genes. The Dicer enzyme breaks down long double-stranded RNA molecules into siRNA; synthetically produced or in vitro transcribed siRNA duplexes can be transfected into cells; and (III) siRNA molecules can be produced in vivo from plasmids, retroviral vectors, or adenoviruses. All three methods are possible. Guidance for nuclease is provided by siRNA, which is integrated into the RISC [47].

Source: https://febs.onlinelibrary.wiley.com/doi/10.1046/j.1432-1033.2003.03555.

Gene function may be studied using antisense drugs to regulate gene expression in cells to cure different disorders, and antisense technology provides a valuable tool for investigating gene function. A precise understanding of the target mRNA sequence and the implementation details of its corresponding antisense agents for the inhibition of its protein message may lead to highly selective and efficient gene silencing of any illness. In the cancer therapy and HIV, as well as other mutating viral infections, this method has proven to be very effective [21]. To do this, the method makes use of many molecules and substances such as antisense oligonucleotides, ribozymes, siRNA, microRNA, and apatamers, amongst others. Despite the immense therapeutic promise of antisense technology, transport of AS ODNs and siRNA to their target sites is often hampered by a number of obstacles. Nanocarrier-associated oligonucleotides and siRNA face different obstacles than those faced by oligonucleotides and siRNA that are uncoated [22]. It is necessary for an antisense drug to travel via circulation to the target tissue, penetrate sick cells, and bind with complementary mRNA after endosomal release in order to suppress protein production and therefore bring about a therapeutic response. However, the enormous size as well as ionic structure of the oligonucleotides and siRNA makes it difficult for them to cross the diverse biological membranes effectively [23]. For effective delivery of AS ODNs, which have likewise been extensively explored for siRNA, we address the numerous hurdles met by these ODNs. AS ODNs and siRNA have the same end goal and activity, therefore the issues, obstacles, and solutions that apply to one also apply to the other [24].

RNAi

An extremely effective tool for researching the fundamental biology of cells, RNA interference (RNAi) allows for the suppression of gene expression in a broad variety of cell types to examine protein function [25]. RNAi was formerly considered a niche approach, but now it is widely accepted as a necessary tool for the research of gene function. Protein knockdown research, phenotypic analysis, functionality recovery, effective decision, *in vivo* reduction, as well as therapeutic target identification have all benefited from this powerful technique in recent years. In RNAi, there are two kinds of tiny RNA molecules. Synthetic small interfering RNA (siRNA) molecules inhibit gene expression by blocking the cleavage of mRNA, which is the initial step. However, a group of naturally occurring 19–22 nucleotide length single-stranded RNA molecules known as microRNAs (miRNAs) is responsible for controlling gene expression by attaching to the 3′ untranslated regions (UTR), which inhibits translation [26].

In vitro dicing, synthetic chemicals, RNAi vectors, and other approaches may all be used to trigger RNAi in cells [27]. Short dsRNA-short interfering RNA-starts the degradation of a particular cellular

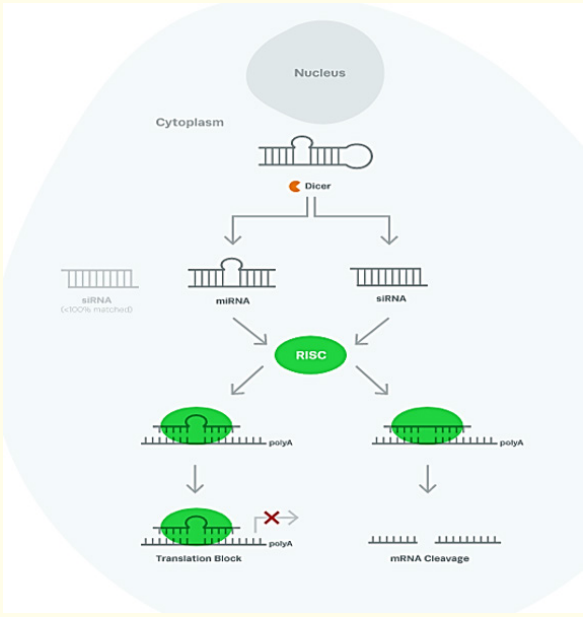

Figure 3: TRANSFECTION AND REPAIR MECHANISM OF RNAi: RNAi mechanism schematic. As an endonuclease, Dicer is capable of degrading long dsRNA or pre-miRs into smaller pieces known as siRNAs or miRNAs. Binding RISC proteins to siRNA/miRNA helps oligos discover the corresponding RNA and cut it, suppressing protein production, as seen in this figure. There is no mRNA cleavage in the event of an incomplete match between the siRNA/miRNA and the target mRNA, but RISC blocks translation [48].

mRNA in mammalian cells. There are many steps involved in this process: First, the siRNA antisense sequence forms part of an RNA-induced silencing complex (RISC), [28] which next locates and cuts off the siRNA-targeted transcript. The cleaved message is then targeted for destruction, resulting in the loss of protein production.

Transcribing genes encoding miRNAs results in the production of lengthy primary transcripts (pri-miRNAs), which are then processed by the RNase III–type enzyme Drosha, resulting in hairpin structures with a length of 70–90 bp" (pre-miRNAs) [29]. miRNA duplexes of 19 to 22 nucleotides in length are formed by the RNase III protein Dicer in the cytoplasm, where they are transported to the nucleus and further processed. The RNA-induced silencing complex (RISC), a multiple protein nuclease complex, recognizes the miRNA duplex and one of the two strands, the guide strand, aids this protein complex in identifying its associated mRNA transcript [30]. It is yet to be completely understood how the RISC-miRNA complex interacts with the 3' UTR of target mRNAs in sites of poor sequence homology, which inhibits protein production [31].

The RNAi (RNA interference) process may be regulated by either siRNA or miRNA, depending on the situation [32]. Both are integrated into the RISC complex after being processed by the enzyme Dicer within the cell (RNA-induced silencing complex). There are, however, minor variations between the two. It is possible to produce siRNA exogenously and then directly transfect it into cells or it may be made within the cell by inserting vectors that generate short-hairpin RNA (shRNA), which is the precursor of siRTs. However, miRNA is a single-stranded RNA that originates in the introns of bigger RNA molecules and arises from endogenous non-coding RNA. However, the cellular RNAi mechanism that normally processes genome-encoded miRNAs, which are crucial for cellular control of gene expression by modifying mRNA stability, translation, as well as chromatin structures, transforms shRNA into functional siRNA [33].

Since miRNA's matching to its mRNA target is imprecise, it may impede translation of a wide range of mRNA sequences [34], while siRNA normally binds properly and specifically to the mRNA target in animals. Instead of only repressing translation, plants' miRNAs feature complementary sequences that cause mRNA cleavage. RNA mediated transcriptional silencing is a mechanism in which both siRNA and miRNA play a role in epigenetics (RITS) [35]. Due to their functions in regulating gene expression, these proteins are attractive therapeutic targets for a variety of reasons.

RNAi is a simple approach for ascribing functions to genes by sequence-specific knockdown of target genes and subsequent phenotypic investigation [36]. There are several benefits to using the RNAi method to silence a specific gene, including great effectiveness in knocking it down, the ease with which it may be targeted and the capacity to silence it for lengthy periods of time. Many questions in cell biology have been answered using this powerful instrument. The fact that RNAi sequences bind to several targets has been established by researchers [37]. The cell's gene expression pattern and, perhaps, its phenotype are altered as a result of these off-target modifications. Such a signature has a significant

potential of causing false-positive results that might jeopardize a project's completion. The knockdown efficiency of even the most recent algorithm-based sequence designs is typically about 80% or less. For example, the consequences of a modest but precise knockdown might be obscured by an off-target signature that obscures phenotypic alterations. These difficulties may make RNAi unpredictable, sluggish, and hazardous, especially in drug development, where speed and dependability of findings are critical.

Site-directed mutagenesis

Specific, targeted alterations in double-stranded plasmid DNA may be made by using site-directed mutagenesis (SDM). Some of the numerous motivations to modify DNA include studying changes in protein function as a consequence of the DNA modification; screening for mutations (at the DNA, RNA or protein level) with a desired feature; and introducing or removing restriction endonucleases or tags. Using custom-designed oligonucleotide primers, SDM is an *in vitro* process that may result in a desired mutation in a DNA plasmid. Kunkel's approach that relies on a strain lacking in the enzymes dUTPase and urea deglycosylase such that the recipient E. coli destroys the wild-type DNA was frequently employed in the past [38].

Many commercial kits now on the market need modifications or unique E. coli strains. By using ordinary primers and inverse PCR, the most commonly used procedures do not need any special strains or modifications. Both an overlapping and back-to-back orientation may be used for these strategies. In the case of overlapping primers, the product will re-circulate and result in a plasmid with two distinct nicks. Circular products like these can be immediately converted into E. coli, albeit at a lesser rate than non-nicked DNA plasmids can. It is possible to convert plasmids that have been nicked using back-to-back primer design approaches, which also allow for exponential amplification to produce substantially more of the target product. Furthermore, since the primers do not overlap, the plasmid and the restrictions of contemporary primer production are the main limits on deletion and insertion sizes. Insertions of up to 100 bp may now be generated in a single step by utilizing two primers to divide the insertion.

Using site-directed mutagenesis, particular mutations may be studied. Some examples: It was used to test the sensitivity of particular species to chemicals often employed in laboratories. For this experiment, scientists employed site-directed mutagenesis to

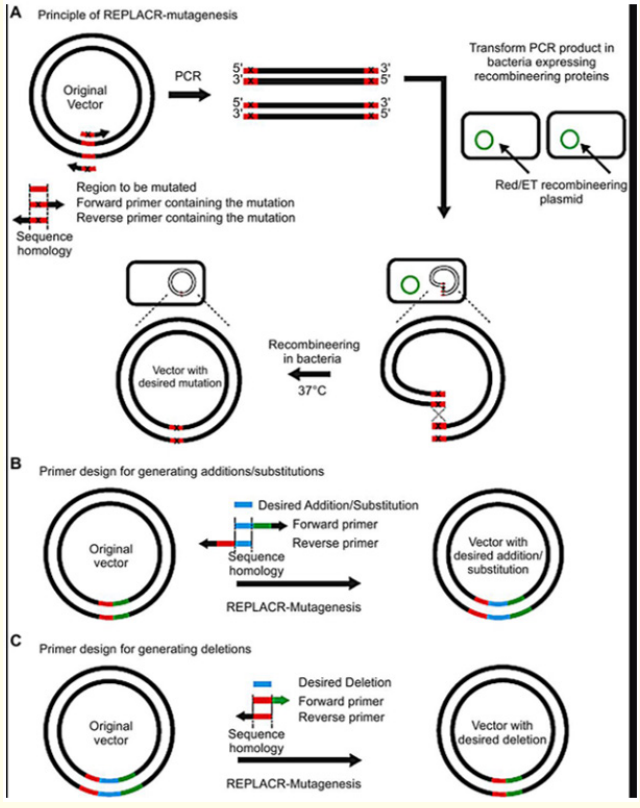

Figure 4: Transfection and Repair Mechanism of Site-Directed Mutagenesis: (A) Recombination may occur when the desired mutation is present in both primers. Recombinant proteins (Redγ, β, α and RecA) are introduced into bacteria that have been transformed with the PCR product. After plasmid extraction, PCR and sequencing indicate the presence of mutations in the plasmid. An example of forward and reverse primers with the necessary alteration is shown in (B). In this example, a sequence next to the sequence to be deleted is included in the forward primer, and the reverse primer comprises a sequence that is homologous to both the forward primer and the adjacent vector sequence [49].

imitate chemical changes. Specific amino acids in the protein were altered as a consequence of the mutation, and the implications of this modification were investigated. Wild type protein is depicted at the top, with M denoting the first amino acid methionine and signifying the end of translation. The following images depict each of the 19 isoleucine 5 mutations known to date. Such approaches as alanine scanning mutagenesis, where residues are systematically altered to alanine in order to discover residues critical to the structure or function of a protein, may be used to implement the site-

directed strategy. Using site saturation mutagenesis, all potential amino acids may be replaced at particular codon sites in one or more genes. Some of the common disadvantages of site-directed mutagenesis include but are not limited to the following: Problematic large insertions and deletions, DNA's low amplification yield, primers that are complementary anneal together.

CRISPR/Cas9

Genome editing technologies include the CRISPR/Cas9 system. Because lengthy synthetic RNAs were costly to produce and not generally accessible when CRISPR system was originally established, it seemed appropriate to build plasmid vectors to generate Cas enzyme and gRNA [39]. It's not always easy to use plasmids for genome editing because of the time-consuming cloning operations required before an experiment can begin. Before beginning a CRISPR experiment, it is necessary to give the plasmids or viruses a few more days or weeks of preparation. Creating a template DNA sequence from which gRNA may be transcribed is the first step in the process. An amplified plasmid must be isolated and transfected into the cells where the CRISPR experiment is to be carried out. For the CRISPR/Cas9 complex to work, effective transport into the nucleus of target cells is essential. DNA plasmids, messenger RNAs, and ribonucleoproteins might all be used to deliver the CRISPR/Cas9 complex. DNA (RNP, Cas9 protein complexed with sgRNA). RNP complex administration without DNA or mRNA eliminates many of the difficulties associated with those methods. Since there is no requirement for internal transcription and translation, RNP delivery allows genome editing to proceed more quickly. The temporary genome editing, on the other hand, decreases off-target consequences, insertional mutagenesis, and immunological responses while allowing for high editing efficiency. It is also possible to improve genome-editing efficiency in a variety of settings by using RNP delivery, which is particularly useful for cells with poor transcription and translation activity. In the CRISPR/Cas sector, RNP delivery is a potential platform because of these features.

Using electroporation to deliver RNPs works effectively for the vast majority of CRISPR investigations in a wide variety of cell types. Delivering a generated RNP to cells has the following advantages: Chemical changes in the Alt-R guide RNAs guard against degradation by the cellular RNase. Upon delivery, the RNP complex is ready to use. The Cas enzyme does not have to wait for the guide RNA to attach to it in the cells since this is done before the cells are delivered. That's because of the short half-life of recombinant Cas9. Off-target cleavage is less of a concern when RNP complexes are used since they reduce the time that Cas9 spends in the cells, which is an important factor in precision genome editing. The protein's lifespan has been slashed as a result of cellular recycling. There will be no plasmid sequences that might have an impact on studies that are not removed from the cells when the experiment is completed. When plasmid DNA is incorporated into the genome, this problem may develop. Nevertheless, plasmids have the advantage of being able to impose selective pressures on cells if one wishes to integrate plasmid DNA into the cells.

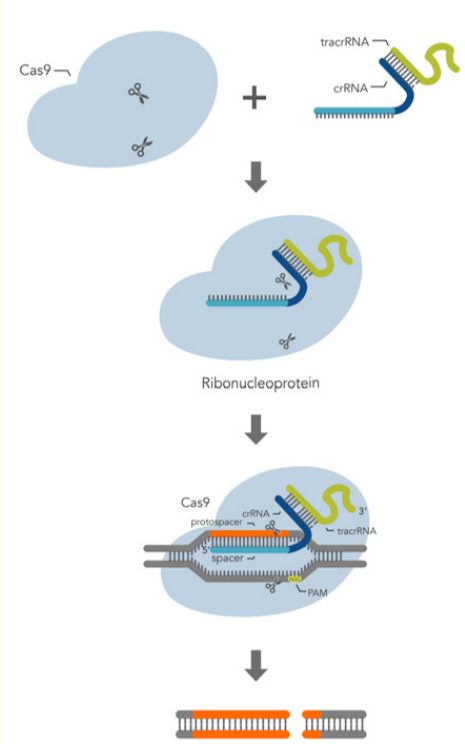

Figure 5: CRISPR-Cas9 gene editing is seen in Figure 6. There are two ways to begin the process of forming RNPs (step 1): in cells or on the bench. As a first step, the two components of the CRISPR (crRNA) and the transactivating CRISPR (tracrRNA) are linked together to produce one full guide RNA. The Cas enzyme then binds to gRNA, creating ribonucleoprotein as a result (RNP). To get RNP into cells, RNP must first be synthesized on the lab bench. The gRNA drives RNP to the target within cells in step 2. It is then hacked into pieces (step 3) [51].

On 7th October 2020, the Nobel Prize in Chemistry was awarded to Jennifer Doudna and Emmanuel Charpentier for their work with the CRISPR/Cas9 system [40]. Many others, notably Virginijus Siksnys, played an important role in advancing gene editing, but their contributions were overlooked in the awarding of the Prize.

In 2008, Danisco used the CRISPR/Cas9 technology for the first time [41]. As a result, the method has been used by a number of food producers to create cheese and yoghurt. DNA in human and other animal cells cultured on petri dishes has been deleted, inserted, and modified ever since. Transgenic animals, such as mice, rats, zebrafish, pigs, and primates, are also being developed with the use of this technology. Using CRISPR/Cas 9, scientists were able to eradicate muscular dystrophy in mice, treat a rare liver ailment, and create human cells that are resistant to HIV as recently as 2014 [42]. Additionally, pluripotent stem cells and transgenic pig organs are being studied to supply human organs. The study hopes to assist alleviate some of the lack of human organs available for transplant procedures and to overcome various negative effects of organ transplantation, such as graft-versus-host disease (GVHD). Insect-borne illnesses, such as malaria and lyme disease, may be eliminated by genetically engineering insects, such as mosquitoes, using this technique.

CRISPR/Cas9 was first used on human embryos in April 2015 by a Chinese team [43]. A huge bioethical controversy has been sparked as a result of this advancement and the reducing prices of the technology. There are two primary difficulties that the technology must deal with.

The first problem is a philosophical one. At issue is whether or whether 'germ-line' cells like eggs and sperm, which are capable of passing genetic information on to the next generation, should be altered using CRISPR/Cas9. While the ability to make designer babies is still several years away, a public discussion has already started on the subject. Some scientists, including those who helped develop CRISPR/Cas9, have asked for a halt on its usage in germline cells because of the considerable worry [44].

Safety is the second concern. One of the biggest issues is that the technology is still in its infancy and understanding of the genome is quite restricted [45]. Researchers warn that further work is needed to improve the technology's accuracy and prevent alterations made to one area of the genome from introducing changes elsewhere that might have unanticipated effects. When it comes to health-related apps, this is a critical concern. Another major concern is that once a changed creature, such as a plant or bug, is released into the environment, it may harm biodiversity since it is difficult to identify from the wild-type.

For CRISPR editing to work, cells must be able to survive plasmid transfection, but even then, there are issues with plasmid usage, such as delays in CRISPR research. Making plasmids takes up important bench time, making this the most evident negative in

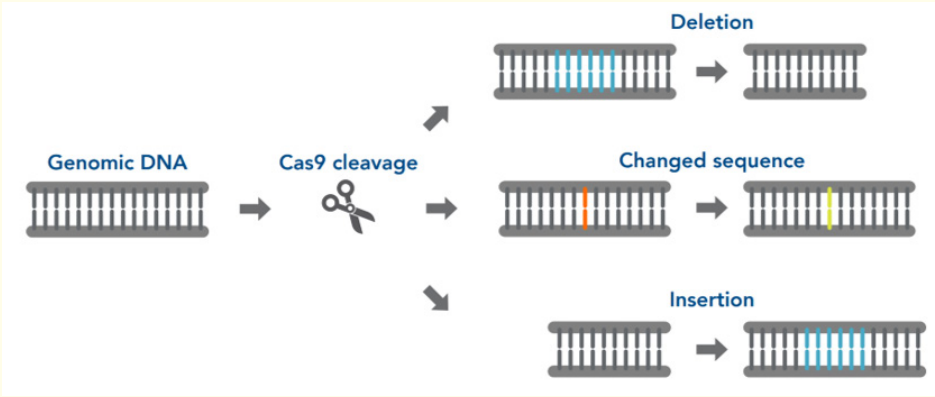

Figure 6: Genomic DNA cleavage may lead to a variety of effects. For example, cellular DNA repair processes such as NHEJ may cause deletions, changes in sequences, and tiny insertions in DNA sequences [52].

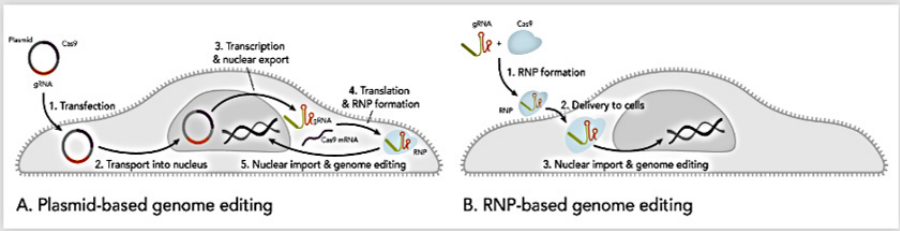

Figure 7: A comparison of what happens when cells are exposed to either a CRISPR plasmid or a ribonucleoprotein. A) When cells are transfected with a CRISPR plasmid, the plasmid enters the nucleus where transcription occurs. (B) This is followed by nuclear export and translation, resulting in Cas enzyme protein. Folding of the newly formed protein follows. The gRNA is also transcribed from the plasmids and exported from the nucleus at this point. In order to do genome editing, the Cas enzyme binds together with the gRNA to create a ribonucleoprotein and returns to the nucleus. (B) The enzyme quickly begins cutting specific genomic DNA when pre-formed RNP is delivered into cells through nucleofection [53].

terms of timeliness. The CRISPR genome editing process must first wait for a plasmid to be transfected. In order for gRNA and Cas-encoding mRNA to be transcribed, a plasmid must first be delivered into the cell's nucleus. Translation of the Cas protein requires the mRNA to be exported from the nucleus. An enzyme known as Cas is involved in the transfer of Cas and the gRNA into nucleus, where editing happens. All of these stages are time-consuming. The procedure may take up to 24hours. Certain operations, including as medication administration and homology-directed recombination, may be hindered due to the inability to predict when the enzyme is produced (HDR). Additionally, plasmids may induce Cas and gRNA to stay active in cells for lengthy periods of time. Off-target consequences are common in CRISPR genome editing research (OTEs). 'OTEs,' or unwanted transposition events, are sequence alterations in genomic DNA that are comparable to the target sequence. OTEs are formed when the Cas enzyme is guided to the incorrect location by the gRNA because of the similarity in sequence. OTEs are less likely to occur when the Cas enzyme is correctly directed to the target site thanks to appropriate gRNA design.

Conclusion

Fundamental alterations in genetics have been brought about through genome editing technology. Site-directed mutagenesis uses a plasmid to introduce a new DNA fragment. To do this, a restriction enzyme must first cleave a spot on the plasmid and then a pair of matching oligonucleotides with the plasmid's desired gene mutation must be ligated together. The process of genetic recombination known as "homologous recombination" involves the exchange of nucleotide sequences between two identical or closely related DNA molecules. It is only via the mRNA that the converted protein peptide sequence is made "sense" with the genetic code. mRNA is synthesized from the "antisense" strand of DNA, which is complementary to "sense" DNA. In cell biology, RNAi is enables the suppression of gene expression in a broad variety of cell types to examine protein function for a short period of time. CRISPR-Cas9 technology stands out and it's the most effective method because its ability to inhibits protein expression removing any confounding effects from residual low levels of protein expression after knockout.

Author Notes

Abdulghani A. Naeem and Saud A. Abdulsamad contributed equally to this work.

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