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Role of Recombinant DNA Technology in Transforming Human Life: A Review

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ABSTRACT

Recombinant DNA technology has revolutionized various aspects of human life, significantly impacting health, agriculture, and environmental sustainability. By enabling the manipulation of DNA from different species, this technology facilitates the production of essential proteins like insulin and growth hormones, enhancing medical treatments for chronic diseases. In agriculture, it promotes the development of genetically modified organisms that exhibit increased resistance to pests and improved crop yields, addressing food security challenges. Furthermore, recombinant DNA techniques are crucial in bioremediation efforts and gene therapy applications, highlighting their multidisciplinary relevance in contemporary society.

Keywords: Recombinant DNA Technology, Human Life, Genetic Transformation.

INTRODUCTION

With the increase in the global population, human existence faces a multitude of challenges. Factors like hunger due to limited food supplies, the spread of infectious diseases, and environmental concerns linked to rapid industrialization weigh heavily on our communities (Khan et al., 2016). Basic human needs, like a clean living environment, access to food, and health services, are crucial. However, as more people inhabit the Earth, the demand for safe and affordable food grows stronger. The World Health Organization (2019) reports that over 8.9 million individuals lose their lives each year due to both communicable and non-communicable diseases, which include pressing issues like COVID-19, heart disease, cancer, tuberculosis, malaria, and HIV/AIDS. Despite various efforts, the global food supply continues to fall short of what is necessary to support everyone, and many healthcare systems in developing nations struggle to meet basic needs. Moreover, the swift pace of industrialization contributes to worsening environmental pollution. Often, industrial waste is dumped into rivers and oceans, harming marine ecosystems and indirectly threatening human health (Kumar et al., 2015).

To counter these trials, it's essential to harness modern technology to foster sustainable food production and a healthier environment. While traditional methods, like selective breeding and herbal medicine, have their place, genetic engineering incorporates advanced techniques like molecular cloning to tackle agricultural, health, and environmental challenges. It requires both time and precision, but it can significantly enhance product quality and dependability (Shinde et al., 2018). Altering plant genomes can happen through methods like homologous recombination or targeted genome modification using specialized enzymes (Gray, 2021). Recombinant DNA technology, also known as gene cloning or genetic engineering, refers to a range of experimental protocols that facilitate the transfer of genetic information (DNA) from

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one organism to another (Trushali et al., 2018). This technology is instrumental in improving health, as it helps develop new vaccines and medications. It also enhances treatment methods by creating diagnostic tools, monitoring systems, and innovative therapeutic strategies. For instance, the production of synthetic human insulin and erythropoietin from genetically modified bacteria and creating experimental mutant mice for scientific research demonstrate the significant role of genetic engineering in healthcare.

On the environmental front, these strategies work to convert waste into biofuels, clean up oil spills, reduce carbon footprints, and detect contaminants like arsenic in drinking water (Trushali et al., 2018). The emergence of recombinant DNA technology has sparked a profound revolution in biological sciences, leading to groundbreaking advancements. It provides new opportunities for generating various therapeutic products swiftly, enhancing medical genetics, and transforming biomedicine by modifying microorganisms, plants, and animals to yield valuable medical substances (Liu et al., 2013).

RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology is advancing quickly, with scientists globally developing new methods, tools, and altered products for a range of fields such as agriculture, healthcare, and environmental protection (Rohan, 2022). Essentially, recombinant DNA technology involves the process of combining DNA from two distinct organisms and placing it into a host organism. This creates new genetic combinations—or genomes—that hold significant value in science, medicine, and various industries, as explained by resources like Britannica.com.

Synthesis of Human Insulin

Recombinant human insulin is notably similar to insulin derived from pigs, and it tends to provoke fewer immune responses in patients. This advancement makes it a more affordable option that can better meet medical demands. Interestingly, human growth hormone was the first protein ever expressed in tobacco plants (Amina et al., 2021). Beyond just insulin, many new therapeutic drugs rooted in recombinant DNA technology have seen remarkable advancements, and various systems for protein production have also been created. Researchers have engineered different microbial strains specifically designed to formulate drugs (Pennisi, 2013). However, the development of molecular medicines based on proteins faces significant challenges, particularly with the methods and biological processes involved in creating medically important substances through recombinant DNA techniques. Therefore, there's an urgent need for enhancing both the quality and quantity of these molecular drugs. While cell factories play a crucial role in recombinant DNA endeavors, they require deeper exploration because traditional factories often fall short of meeting current demands (Khan et al., 2016).

Moreover, the engineering of oncolytic adenoviruses using factors like endothelial growth factor and Notch signaling has shown potential as a targeted treatment for breast cancer. This approach disrupts tumor angiogenesis, effectively reducing blood vessel numbers and leading to significant changes in the tumor's vascular structure, which improves treatment efficacy (Urban and Roth, 2015). Significant strides have also been made in modifying the influenza virus genome with recombinant DNA technology to improve vaccine development. This modification typically involves engineering vectors to express foreign genes. For instance,

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researchers replaced the NS gene of the influenza virus with a foreign gene, such as chloramphenicol acetyltransferase. Once the RNA is recombined, it is expressed and packaged into viral particles after being transfected with purified influenza A virus alongside a helper virus. It's been noted that the terminal bases from the influenza A virus RNA are essential for signaling RNA replication, transcription, and proper packaging (Suleiman et al., 2016).

The innovative production systems significantly enhance the development pipelines for various vaccines and drugs. Achieving high-quality protein production hinges on the cellular physiology and the conditions under which these cells operate. When cells experience stress, protein expression can be inhibited, yet certain stressors may actually boost production in some instances. Therefore, continuous improvements are essential for safer genetic and metabolic production practices. Microorganisms are viewed as efficient hosts for the production of molecular medicines due to their ability to incorporate foreign genes with minimal resistance and the ease of controlling their expression. Compared to plant and mammalian cells, microbial systems present a simpler production mechanism, which leads to better protein yield and quality. While common microbial species like bacteria and yeast show great promise, less common strains have also emerged as potential cell factories for producing recombinant molecular drugs (Khan et al., 2016).

PROCEDURES INVOLVED IN RECOMBINANT DNA TECHNOLOGY

A recombinant DNA molecule is created by piecing together segments from two or more different DNA sources. In the right conditions, this recombinant DNA can enter a cell and replicate, either independently or after becoming part of a chromosome. To manipulate an organism's genetic makeup, scientists can add new genes and regulatory elements, or they can mix genes and regulatory elements to decrease or halt the expression of existing genes (Bazan-Peregrino, 2013). The process starts with enzymatic cleavage, where restriction endonucleases are used to cut DNA at specific target sequences, generating various DNA fragments. DNA ligase is then utilized to link these fragments together, securing the desired gene in a vector. After the vector is formed, it gets introduced into a host organism, which is cultivated to produce numerous copies of the DNA fragment embedded in it. Finally, researchers select and harvest clones that contain the relevant DNA fragments (Venter, 2007).

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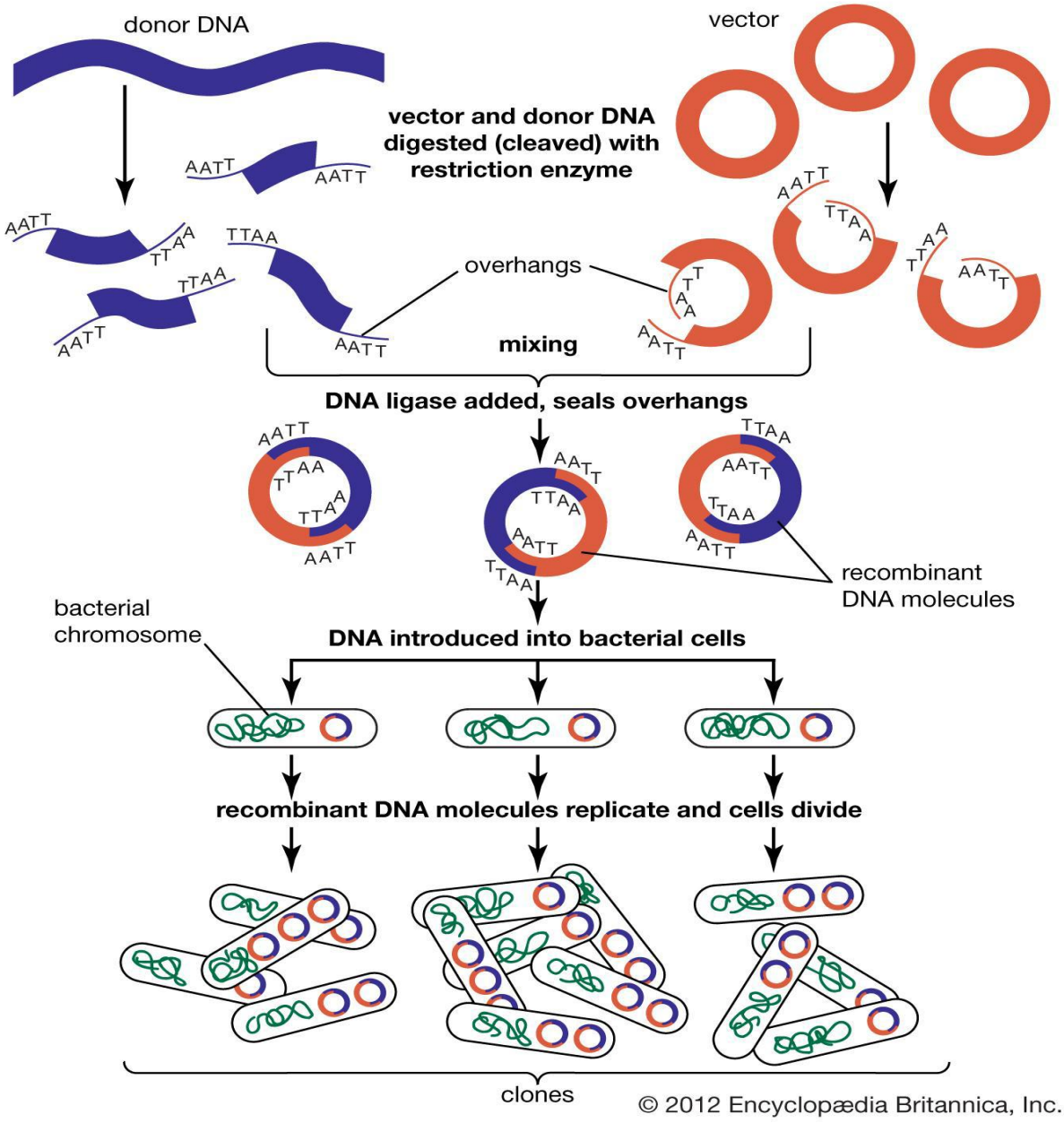

Figure 1: Summary of Recombinant DNA technology/cloning procedure.(Kumar, D'Souza, & Asthana, 2018)

APPLICATION OF RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology has proven invaluable across several fields, leading to targeted enhancements in areas like crop farming, pharmaceuticals, gene therapy, vaccine development, and bioremediation. Specifically, bioremediation serves as a waste management strategy that employs either naturally occurring or intentionally engineered genetically modified organisms (GMOs) to eliminate environmental pollutants. The process involves breaking down harmful substances into less toxic or even non-toxic

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compounds, effectively cleaning contaminated soil and water in a thorough, quick, and cost-effective manner (Gray, 2021). In the realm of biotechnology, medicine, and research, recombinant DNA has widespread applications. Nowadays, recombinant proteins and other products from DNA technology can be found in nearly every pharmacy, clinic, veterinarian's office, medical testing facility, and biological research lab across the West. Furthermore, genetically engineered organisms and their derivatives have made their way into various sectors, including farms, grocery stores, home medicine cabinets, and pet shops, which may offer genetically modified animals like Goldfish (Khan et al., 2016).

In Food and Agriculture

The development of genetically modified crops in agriculture, aimed at enhancing yield and improving resistance to pests or herbicides, has gained some level of public acceptance and is already commercially practiced in various countries (Khan et al., 2016). One notable example is the tomato CGN-89564-2, which was the first genetically modified crop product to be licensed for human use. Created in 1994, it was designed to delay softening of tomato flesh, helping to reduce post-harvest losses (Khan et al., 2016). Another important case is Golden rice, a genetically engineered variety that expresses enzymes responsible for β-carotene biosynthesis. This rice aims to combat vitamin A deficiency, a significant health issue for many people worldwide. However, Golden rice is not yet in circulation due to ongoing regulatory and intellectual property challenges (Khan et al., 2016). Herbicide-resistant crops have also emerged, with commercial varieties of essential crops like soybeans, maize, sorghum, canola, alfalfa, and cotton incorporating recombinant genes that confer resistance to glyphosate, a widely used herbicide. This technology simplifies weed management through glyphosate application and is in common use across several countries (Kumar, 2015). Insect-resistant crops represent another innovative application.

The bacterium *Bacillus thuringiensis* naturally produces a protein, known as Bt toxin, which is effective against certain insects. This bacterium has long been used as an insect control method, and recently, scientists developed plants that express a recombinant version of this protein, providing an effective means to deter specific insect pests. However, environmental concerns regarding these transgenic crops remain to be fully addressed (Cardi et al., 2016). Lastly, recombinant chymosin plays a key role in cheese production. It was the first genetically engineered food additive used commercially and is essential for making cheese. Traditionally, chymosin was obtained from rennet sourced from the fourth stomach of milk-fed calves. However, scientists have engineered a non-pathogenic strain of E. coli, known as K-12, for the large-scale laboratory production of this enzyme, revolutionizing cheese manufacturing**.**

In Medicine

In medicine, drug delivery systems that rely on bacterial or viral hosts can pose serious risks, particularly if the organism shows genetic instability and transforms into a pathogenic variant or if there are issues with incomplete purification. A notable example from agriculture is the use of the soil bacterium Agrobacterium tumefaciens as a gene transfer vehicle. Despite its effectiveness, it is also known for generating tumors and causing crown gall disease in eudicot plants. Concerns about genetic reversions are significant when it comes to gene therapy, especially for treating or preventing genetic disorders and acquired diseases that currently have no cure.

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As research transitions from laboratory settings to clinical trials, finding a safe and effective method to deliver a targeted altered gene has become crucial. With the progress of recombinant DNA technology, numerous therapeutic agents and methods have been discovered and are now being utilized around the world, including applications in gene therapy and advanced treatments for previously untreatable conditions;

- ✓ **Recombinant Human Insulin**: This form of insulin has largely replaced insulin derived from animal sources, such as pigs and cattle, for managing insulin-dependent diabetes. Various recombinant insulin formulations are widely used today. The production involves inserting the human insulin gene into microorganisms like *E. coli* or yeast (*Saccharomyces cerevisiae*), which then synthesize insulin for medical purposes.
- ✓ **Recombinant Human Growth Hormone (HGH, Somatotropin)**: This is prescribed to individuals with pituitary gland deficiencies that hinder normal growth and development. Previously, HGH was extracted from cadaver pituitary glands, a method linked to Creutzfeldt–Jakob disease. Recombinant HGH has eliminated this risk and is now the standard treatment. However, it has been misused by athletes and others for performance enhancement.
- ✓ **Recombinant Blood Clotting Factor VIII**: This protein is used to treat hemophilia patients who cannot produce sufficient Factor VIII for normal blood clotting. Before recombinant technology, Factor VIII was isolated from pooled human blood, posing significant risks of transmitting infections like HIV and hepatitis B. Recombinant Factor VIII has greatly reduced these risks.
- ✓ **Recombinant Hepatitis B Vaccine**: This vaccine is crucial for controlling hepatitis B infections. It contains a hepatitis B virus surface antigen produced in yeast cells. The recombinant subunit vaccine was essential because the hepatitis B virus cannot be cultured in vitro, unlike viruses such as polio.
- **Recombinant Antibodies (rAbs)**: These antibodies are generated in vitro using mammalian cell-based expression systems. Their ability to bind specifically to a single epitope makes them valuable for both research and therapeutic applications, including treatments for cancer, infections, and autoimmune diseases.
- **Diagnosis of HIV Infection**: The three primary methods for diagnosing HIV rely on recombinant DNA technology. The antibody test (ELISA or western blot) uses recombinant HIV proteins to detect antibodies produced in response to the virus. The DNA test identifies HIV genetic material using reverse transcription polymerase chain reaction (RT-PCR), made possible by the molecular cloning and sequencing of HIV genomes.

Application of genetic engineering in bioremediation:

In bioremediation, bacteria like Pseudomonas putida and Nitrosomonas europaea are commonly employed for their ability to clean up environmental contaminants. Numerous studies have shown how effective these microorganisms can be in restoration projects. For instance, researchers from the University of California found that Pseudomonas putida could break down toluene and xylene compounds in contaminated soil, leading to a remarkable 85% reduction in pollutant levels within just 30 days. Similarly, at TU Berlin, Nitrosomonas europaea was successfully utilized to treat ammonia-laden wastewater from agricultural runoff, converting harmful ammonia into safer nitrates with over 90% efficiency. A collaborative effort between Chinese and Canadian scientists demonstrated even more impressive results by employing

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both bacteria in a dual-biofilm reactor system. This innovative setup allowed for the simultaneous degradation of aromatic hydrocarbons and the removal of excess nitrogen compounds from industrial effluents, showing better results than relying on a single type of bacteria. The goal is to isolate the original genes in these bacteria that facilitate bioremediation and then manipulate those genes for introduction into a suitable host, such as E. coli. However, caution is warranted since this could affect natural ecosystems; genetically modified bacteria with enhanced abilities to break down petroleum might inadvertently disrupt critical petroleum product balances. Consequently, careful monitoring during bioremediation efforts is crucial. When generating genetically modified bacteria, a straightforward testing method involves incorporating a marker gene, often one that confers antibiotic resistance. While this can help identify successfully modified organisms, it can also lead to the unintended emergence of antibiotic-resistant bacteria if not managed properly, potentially posing a threat to natural environments (Trushali et al., 2018).

Application of Genetic engineering in forensic exercise

A notable biotechnological achievement is the creation of genetically modified fluorescent zebrafish, Danio rerio, along with similar species that possess genes for glowing traits. These fish, marketed under the GloFish patent in the US, come in bright colors like red, green, orange-yellow, blue, and purple. They are sold as pets for indoor aquariums where their vibrant hues can be appreciated in a controlled environment. In the realm of forensic sciences, molecular biology plays a crucial role, heavily relying on DNA analysis to distinguish individuals from samples such as hair, blood stains, and other items collected from crime scenes. This kind of analysis, popularly referred to as genetic fingerprinting, is more accurately termed DNA profiling today. Such techniques are also instrumental in identifying criminals and exploring familial relationships, a process known as kinship analysis, which is commonly applied in paternity testing (Trushali et al., 2018). A highlight in the use of DNA profiling was in the case of the "BTK Killer" from Kansas. Dennis Rader, the infamous BTK (Bind, Torture, Kill) killer, was captured and convicted in 2005 thanks to DNA evidence.

Investigators accessed Rader's daughter's DNA from a routine Pap smear done at her university clinic and compared it to DNA evidence collected from crime scenes over the years. The partial match between the daughter's DNA and the evidence helped highlight a familial connection, ultimately leading to Rader's identification as a suspect and subsequent arrest for multiple murders committed from 1974 to 1991. When it comes to crime detection, it's almost certain that a person commits a crime while leaving behind some trace of DNA. Elements like hair, blood spots, and even routine fingerprints can carry enough DNA to be analyzed through techniques like polymerase chain reaction (PCR). In recent times, this has enabled law enforcement to solve past cases, bringing criminals to justice based on DNA tests conducted on archived materials. Genetic fingerprinting relies on the concept that identical twins are the only individuals with identical genomes, whereas the human genome has variations. Most humans share a similar genome structure, but there are polymorphic sites that include restriction fragment length polymorphisms (RFLPs), short tandem repeats (STRs), and single nucleotide polymorphisms (SNPs) used as vital DNA markers.

Genetic fingerprinting through hybridization probing was one of the first methods for using

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DNA analysis to identify individuals. This technique exploits variations in specific sequences within the human genome known as hypervariable dispersed repetitive sequences, which have different genomic locations across individuals. To create a genetic fingerprint, a DNA sample is treated with a restriction endonuclease, and the fragments are separated via agarose gel electrophoresis, followed by a Southern blot. A labeled probe that binds to these repeat sequences reveals distinctive bands, with each band representing a different restriction fragment. If the procedure is repeated with another individual's DNA, a distinct pattern emerges, demonstrating the variability in the insertion sites of these repeat sequences. Additionally, DNA analysis can be utilized for sex identification. By detecting the presence of a Y chromosome, it becomes possible to differentiate between males and females. This technique can also assist in determining the sex of an unborn child. While conventional methods usually assess sex later in pregnancy based on anatomical differences, DNA tests can provide earlier insights. This can be particularly relevant if there is a hereditary condition associated with male offspring, prompting parents to make crucial decisions about the pregnancy earlier on.

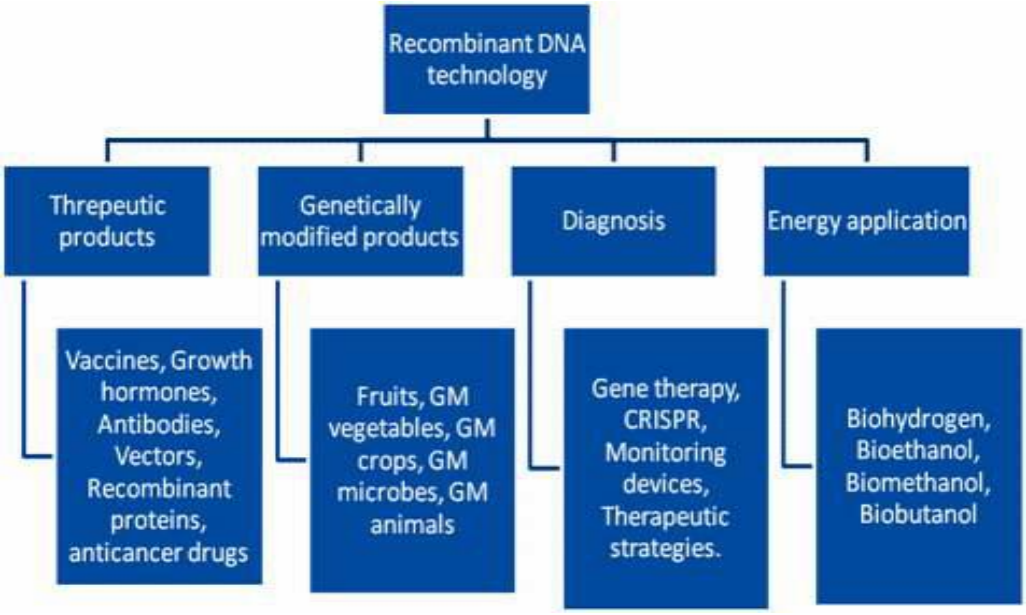

Figure 2: Summarized Illustration of various applications of recombinant DNA technology.

RECENT ADVANCEMENT IN THE APPLICATION OF RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology is advancing quickly, with researchers worldwide working on new methods, devices, and modified products for different areas like agriculture, healthcare, industry, and environmental applications. For instance, Lispro insulin, also known as Humalog, stands out as a rapid and effective recombinant version compared to traditional human insulin (Suleiman et al., 2016). Furthermore, Epoetin alfa is another significant recombinant protein known for its effectiveness in treating anemia. An exciting development in this field includes the use of recombinant hemoglobin H, which has been helpful for children who struggle to produce

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enough hemoglobin H. Additionally, a recombinant form of the cytokine myeloid progenitor inhibitory factor-1 (MPIF-1) achieved great recognition, receiving approval from the Food and Drug Administration (FDA) in December 1997. This innovation has the potential to reduce side effects associated with anticancer drugs by mimicking the activity of vital immune cells (Rohan, 2022). Looking ahead, current research trends in life sciences indicate that the applications of recombinant DNA and gene cloning will continue to be incredibly beneficial. The roster of new and improved therapeutics enabled by recombinant DNA technology keeps expanding. Enhanced human insulin for diabetics, Epoetin alfa for those with anemia, and human growth hormone for children with deficiencies are just a few examples showcasing the ongoing progress in this field. These advancements provide a glimpse into the future potential of recombinant DNA technology.

Clustered Regularly interspaced Short Palindromic Repeats (CRISPR)

The 2020 Nobel Prize in Chemistry was awarded to Emmanuelle Charpentier and Jennifer Doudna for creating the CRISPR/Cas9 gene editing technology, which has allowed for precise modifications of genes (Zhang et al., 2021). This CRISPR-Cas9 system relies on two main components that enable changes to DNA. First, there's an enzyme known as Cas9, acting like molecular scissors that cut both strands of DNA at specific spots in the genome. This allows for the addition or removal of DNA fragments. The second component is called guide RNA (gRNA), made up of a small RNA sequence designed to lead Cas9 to the exact location in the DNA. This RNA has a complementary sequence to the target DNA, ensuring that it binds only to the intended area of the genome. Once the guide RNA has directed Cas9 to the correct location, the enzyme makes a cut through the DNA strands. The cell then detects this damage and attempts to repair it. Remarkably, in just a short time, CRISPR/Cas9 has become a vital and widely used tool for gene editing, impacting many fields, particularly in agriculture, biotechnology, and biomedicine. Its most significant effects are seen in cancer research, where the technology has provided new insights into tumor formation and progression (Zhang et al., 2021).

As a recent advancement in recombinant DNA technology, CRISPR has offered solutions to various challenges across species. It's particularly effective in targeting genes in human cells, allowing for the activation, suppression, addition, or deletion of genes not only in humans but also in mice, rats, zebrafish, bacteria, fruit flies, yeast, nematodes, and crops. This capability has accelerated studies of human diseases in mouse models, simplifying the exploration of gene interactions by enabling modifications of multiple genes at once (Pennisi, 2013). Interestingly, the CRISPR system found in H. hispanica can adapt efficiently to nonlytic viruses. The associated Cas operon encodes nucleases and other proteins that help in this process. Engineering strains with primed CRISPR facilitates the production of crRNAs and acceptance of new spacers. This system integrates new spacers into its locus, generating adaptive immunity (Wang et al., 2016). By recognizing and cleaving foreign DNA or RNA in a sequence-specific manner, the host can store information about intruder genetic material through the integration of photo-spacers. Cas9t functions as DNA endonucleases that utilize RNA for recognizing specific targets (Khan et al., 2018). The Class 2 CRISPR-Cas system, which features single protein effectors, is often employed in genome editing. A crucial variant, known as Dead Cas9, plays a role in recruiting

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histone modifying enzymes and can be used for transcriptional repression, localization of fluorescent labels, and transcriptional activation. CRISPR-induced mutations allow for the targeting of genes involved in creating homozygous gene knockouts, enabling the analysis of essential genes, which can lead to the discovery of new antifungal targets. The natural immunity offered by CRISPR-Cas systems has also been harnessed to create strains resistant to various disruptive viruses (Mohanraju et al., 2016)..

Mechanism of Action of CRISPR

The CRISPR-Cas system, a unique adaptive immune mechanism in prokaryotes, comprises a CRISPR locus characterized by short repetitive sequences interspersed with unique spacers. This array is preceded by an AT-rich leader sequence and is flanked by *cas* genes that encode Cas proteins (Hille and Charpentier, 2016). In *Escherichia coli*, the Cas1 and Cas2 proteins facilitate the acquisition of new spacers through the formation of a complex. The process of interference and spacer acquisition relies on the presence of a protospacer adjacent motif (PAM), ensuring that target sequence selection is not random. Following the transcription of the CRISPR array into a long precursor crRNA, the invader's genetic information is memorized. During the final stages of the immune response, the target nucleic acid is degraded via interference. Specific recognition mechanisms prevent the system from targeting the host genome (Hille and Charpentier, 2016). In *Sulfolobus* species, CRISPR loci often contain multiple spacers that match sequences of conjugative plasmids, which themselves may harbor small CRISPR loci. Active viral DNA replication influences spacer acquisition in these species, and the formation of DNA breaks at replication forks stimulates this process (Liu et al., 2016).

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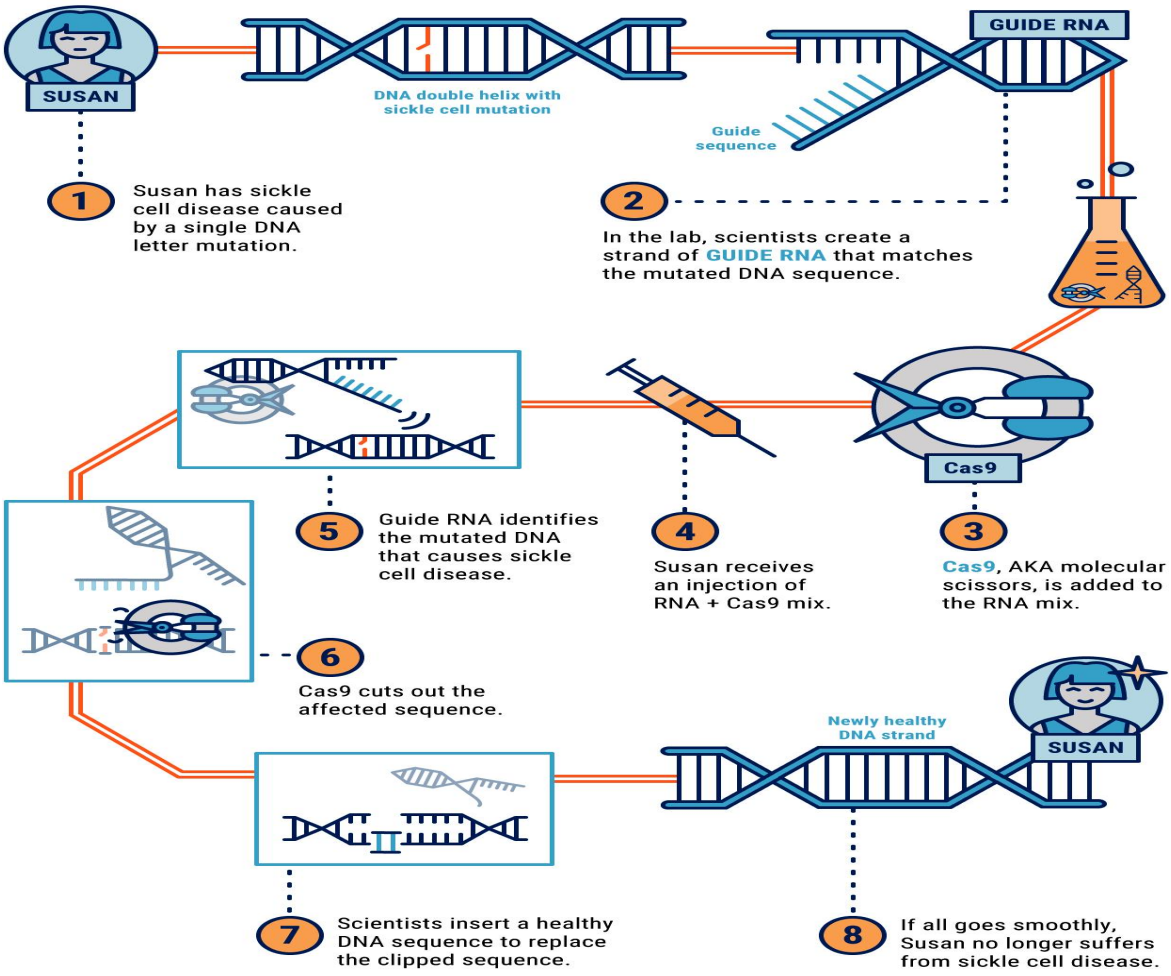

Figure 3: An illustration of how CRISPR work(Liu, Li, Zhu, & Lu, 2024).

CONCLUSION

Recombinant DNA technology has really made a significant impact on our lives. It has evolved and introduced new strategies for biomedical applications, including cancer treatment, managing genetic disorders, diabetes care, and addressing various plant issues, particularly those caused by viral and fungal infections. There's a strong acknowledgment of its role in improving the environment too, through methods like phytoremediation and microbial remediation. Furthermore, this technology has been crucial in enhancing plants' resistance to various challenges, such as drought, pests, and infections, as well as salt stress.

Thereforerecombinant DNA technology has been regarded one of the best and fast growing technology across the globe playing a vital role in improving the quality of man's life.

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