CRISPR CHRONICLES

INTRODUCTION:

The centrality of DNA, genomes, and CRISPR-Cas systems to the biotechnology revolution cannot be overstated. This project aims to explore the mechanism and applications of the CRISPR-Cas systems.

PART ONE: CRACKING THE CODE- COMPREHENSION of CRISPR

Overview of DNA and RNA:

DNA and RNA are cellular molecules at the core of genetic information.

The Watson and Crick model of DNA, proposed by James Watson and Francis Crick in 1953, describes the structure of DNA. It is a double helix structure composed of two strands held together by hydrogen bonds between complementary base pairs (nitrogenous bases). The four bases present in DNA are adenine (A), thymine (T), cytosine (C), and guanine (G). Adenine pairs with thymine (A-T), and cytosine pairs with guanine (C-G).

RNA is a single-stranded nucleic acid molecule, shorter than DNA. RNA uses the same four bases as DNA (A, U, C, and G), but thymine (T) is replaced by uracil (U) in RNA.

The primary difference in the function of the two is that DNA is responsible for storing and transmitting genetic information, while RNA assists in decoding and expressing that information by serving as a template for protein synthesis and carrying out various regulatory functions.

Transcription is the process of synthesis of RNA (ribonucleic acid) molecules using a DNA (deoxyribonucleic acid) template. It is an essential step in gene expression, where the genetic information encoded in DNA is converted into RNA molecule, known as messenger RNA (mRNA). This is processed and transported to the cytoplasm, where protein synthesis occurs. In protein synthesis, the mRNA is read by ribosomes, which assemble amino acids in the correct order according to the mRNA sequence, forming a polypeptide chain that folds into a functional protein.

Protein synthesis is crucial because proteins are the primary building blocks of cells and perform enzymatic catalysis, structural support, transportation, communication, and regulation of cellular processes. Proteins are essential for the growth, development, and activities.

Genomes are not molecules, but the entirety of an organism's genetic material, composed of genes, which are segments of DNA, organized into chromosomes, structures thus containing DNA and associated proteins. Genomes can be composed of DNA in most organisms or RNA in certain viruses.

Application of genetic study:

Science stands to benefit from the trifecta, of genomes, biotechnology, and CRISPR-Cas, whether it be gene editing via precise changes to DNA or the creation of newly engineered organisms and species with completely novel genes. The integration of sequencing technologies into research procedures has enabled scientists to gain access to an organism's entire gene expression profile and thereby better understand how genes interact with one another. We thus find ourselves at the cusp of a great evolutionary leap forward; made possible by harnessing the power of DNA, genomes, and CRISPR-Cas systems. Viruses influence evolution and ecology of life on Earth by acting as predators and genetic exchangers.

Hosts have two types of immunity against these intruders: innate and adaptive systems (B and T cells in humans). Adaptive immune systems have been observed in prokaryotes (unicellular organisms lacking a distinct nucleus and other membrane-bound organelles, bacteria, and archaea) through a system based on CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) which targets DNA or RNA to defend themselves against viruses and other mobile genetic elements. This feature is present in about 84% of archaea and 45% of bacteria. Archaea, while also a group of single-celled microorganisms, differ from bacteria in their genetic and biochemical characteristics, thriving in extreme environments and contributing to ecological processes.

To summarise, scientists are gaining knowledge from bacteria, specifically by discovering and studying the prokaryotic evolutionary adaptation called the CRISPR-Cas system and applying it in ways that can positively impact humanity.

Overview of CRISPR-Cas- the genetic memory system for prokaryotic cells:

In the CRISPR-Cas system, CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats." and CAS stands for "CRISPR-associated." CAS refers to a group of proteins that work together with the CRISPR system to protect bacteria from viruses and other invaders. CAS proteins are soldiers that help bacteria use the CRISPR shield effectively.

The CAS proteins, derived from the genomes of bacteria and archaea play a crucial role in the CRISPR defence mechanism.

When a bacterium encounters a virus, it captures a small piece of the viral DNA and incorporates it into its own genome in a specific region which is called the CRISPR locus. This captured DNA fragment is known as a "spacer." The CRISPR locus is like a filing cabinet where the bacterium keeps a collection of files of spacers, each representing a different virus or foreign invader it has encountered in the past.

The CRISPR locus also consists of repetitive sequences called "repeats" that flank the spacers. These repeats are like the labels or dividers in the filing cabinet, helping to organize and separate the spacers. The repeats are identical or nearly identical to each other and serve as recognition sites for the CRISPR system.

When the bacterium encounters the same virus or invader again, the CRISPR system is activated. The CRISPR-associated (CAS) proteins bind to the CRISPR locus and use the information stored in the spacers to target and cleave the invading DNA, effectively neutralising the infection. The bacterium has a library of information about the different viruses it has encountered, and it can quickly refer to this library to identify and eliminate the specific virus with the help of its Cas proteins.

Scientists have studied and harnessed this capability of CAS proteins to develop powerful gene-editing tools, such as CRISPR-Cas9. These tools allow for precise targeting and modification of specific genes, opening new possibilities for genetic research, medicine, and other applications.

Theory of Horizontal Gene Transfer:

Prokaryotes are unicellular organisms lacking a nucleus and membrane-bound organelles. Unlike eukaryotes, they lack a sophisticated adaptive immune system, making the discovery of adaptive gene mechanisms against infections unexpected in prokaryotes. Prokaryotes primarily rely on innate immunity, utilizing physical barriers, antimicrobial compounds, and restriction-modification systems. They lack the ability to generate diverse specific immune responses or undergo rapid evolutionary adaptations in response to infections. An important mechanism that aids prokaryotes in acquiring new genetic traits, including defence mechanisms against infections, is horizontal gene transfer. It is a process by which genetic material is transferred between different prokaryotic cells, independent of reproduction.

There are three major types of horizontal gene transfer: transformation (uptake of free DNA from the environment), conjugation (direct transfer of DNA between neighbouring cells), and transduction (transfer of DNA via viral vectors). Viral vectors transfer genetic material by infecting host cells and incorporating the desired genetic material into the host's genome or utilizing their replication machinery to produce copies of the genetic material, which can then be transferred to other cells.

The connection between horizontal gene transfer and CRISPR lies in the prokaryotic adaptive immune system of CRISPR-Cas. Horizontal gene transfer can introduce new spacer sequences into prokaryotes, enabling them to acquire immunity against novel viral strains. Prokaryotes can obtain spacers from other prokaryotes that have encountered and successfully defended against specific viruses. Through horizontal gene transfer, prokaryotes can expand their CRISPR-Cas immune repertoire, enhancing their ability to combat infections and adapt to changing viral threats. Horizontal gene transfer is thus a vital process in prokaryotes that allows the acquisition of new genetic traits, including defence mechanisms against infections.

Functions of components of the antiviral defence of CRISPR-Cas:

The CRISPR-Cas defence mechanism is triggered when an invading virus tries to insert its own genetic material; this triggers a three-step process that records the presence of the virus and then destroys it. The first step is adaptation, where new spacers are added into the CRISPR locus, which acts as a chronological record of all viruses encountered by cells throughout generations. In the second stage—expression—the Cas genes and the CRISPR RNA are transcribed. The pre-CRISPR RNA is then processed to create mature CRISPR RNA before interference—the final stage—where target nucleic acid is recognized and destroyed.

<u>CRISPR loci:</u> CRISPR loci, found in a wide range of species, contain repeating sequences that are usually between 21 and 48 base pairs (nitrogenous bases) in length and spacers from 26 to 72 base pairs. CRISPRs can make up a significant portion of a genome, such as Sulfolobus tokodaii str. 7 (with 5 CRISPRs and 458 spacers) and Methanocaldococcus sp. FS406-22 (with 18 CRISPRs and 191 spacers). There may be multiple or single CRISPR loci present,

and while some have adjacent Cas genes/Cas proteins, others rely on trans encoded factors. Leader sequences have also been found in many species. A leader sequence is a nucleotide sequence found before the CRISPR array of interest in the linear arrangement of DNA or RNA, evolutionary in origin and inherited from generation to generation. These sequences serve a s a guide for the CRISPR system to initiate transcription of CRISPR RNA or crRNA. crRNA is a molecular tool that performs the crucial function of recognizing and binding to the target sequences of foreign DNA or RNA. Once bound to a complementary target sequence, crRNA guides the Cas proteins to the target, enabling DNA cleavage or gene regulation, depending on the CRISPR system variant.

Cas proteins: Cas proteins are a highly diverse group of nucleases, helicases, and RNA-binding proteins, involved in adaptation and found in most CRISPR-Cas systems.

They are classified into Type I-A through Type I-F based on the presence of signature protein Cas3, responsible for both helicase and DNase activities.

Cascade Complex: The Cascade system, also known as the CRISPR-associated complex for antiviral defence, is composed of multiple protein subunits including Cas proteins (CasA, CasB, CasC, CasD, and CasE), crRNA, short specific DNA motifs called Protospacer-adjacent motif (PAM) and additional Cas proteins. The specific composition of the Cascade complex can vary slightly depending on the bacterial or archaeal species.

The Cascade complex, functions as a surveillance and targeting system in the Type I CRISPR-Cas immune response: binds crRNA to locate the target, enhances spacer acquisition in some cases, and processes crRNA in most variants. A recent addition is the Type IV system with several cascade genes but no CRISPR or cas1/cas2, guided by protein-DNA interaction as an innate immune defence.

The CRISPR-Cas system is divided into three types - I, II and III. Type I systems encode Cas3 for the Cascade complex, type II encode Cas1 and Cas2, while type III encode Cas10 which typically targets DNA or RNA. Types I and III have been found in both bacteria and archaea, whereas type II has only been identified in bacteria. Type II systems are further subdivided into A, B and C, with A possessing a csn2 gene involved in adaptation, and B containing a cas4 gene responsible for the same process. Type C is unique as it does not contain a fourth gene.

Evolutionary analysis suggests that the Type IV system may have originated from an ancestral innate immune system that gained adaptive abilities by associating with a transposon-like (segments of DNA that can move or transpose within the genome of an organism) element containing cas1 and cas2. The formation of CRISPR repeats occurred through duplication and addition of spacers via the action of Cas1, eventually giving rise to the Type I and III systems. Type II systems, on the other hand, likely formed through the replacement of cascade genes (a series of genes that are activated sequentially, with each gene in the series regulating the expression of the next gene in the pathway) by cas9. Interestingly, Cas9 shares similarities with transposon-encoded proteins.

Phases of activation

Adaptation phase: Adaptation in CRISPR systems is the phase that establishes genetic memory required for subsequent expression and interference phases. During this phase, new spacers are inserted into the CRISPR array, either when encountering a new invader (naïve acquisition) or when there is a pre-existing record of the invader in the CRISPR (primed acquisition). The process involves protospacer selection, generation of spacer material, integration of the spacer into the CRISPR array, and synthesis of a new repeat. Cas1 and Cas2 proteins play a crucial role in spacer integration, with Cas1 responsible for nicking the CRISPR array and integrating the new spacer. Additional factors such as Cas9, Csn2, tracrRNA (in Type II-A): trans-activating CRISPR RNA which is a small RNA molecule that assists in the processing and maturation of crRNA, and Cas4 (in Type I-B) are also involved in spacer acquisition. Spacer selection is guided by certain sequence elements, including the protospacer adjacent motif (PAM), which helps discriminate between self and non-self-sequences.

Expression phase: Expression of CRISPR RNA and Cas genes involves the transcription of the CRISPR-Cas loci to generate RNA-protein guide complexes. The transcription initiates in the leader region, which contains promoter elements and binding sites for regulatory proteins. A long primary transcript called pre-crRNA is generated, which is processed into smaller units corresponding to a single spacer flanked by partial repeats. The processing mechanism varies between different CRISPR-Cas subtypes. Type I and III systems use Cas6 proteins for pre-crRNA processing, while Type II systems depend on host RNase III and a trans-encoded small RNA (tracrRNA). Cas proteins and the crRNA form a CRISPR ribonucleoprotein (crRNP) complex, which plays a role in interference.

Interference phase: Interference is the process by which CRISPR-Cas systems degrade the target DNA or RNA using specific Cas nucleases. The crRNA, bound to Cas protein(s), guides the complex to the corresponding protospacer in the target, triggering its degradation. The type-specific Cas nucleases involved in interference vary between different CRISPR-Cas subtypes. In Type I systems, Cascade locates the target DNA, and Cas3 nuclease/helicase is required for interference. Type II systems only require the Cas9 protein for interference, along with a tracrRNA that base pairs with the pre-crRNA. Type III systems have not yet identified the specific nuclease involved in interference. Interference requires the presence of a PAM sequence and perfect protospacer-crRNA complementarity in the seed region adjacent to the PAM. Cascade and Cas9 undergo conformational changes upon binding to the target, leading to recruitment of the respective nucleases for target degradation.

These three phases of CRISPR systems—adaptation, expression, and interference—form the fundamental steps in the immune response of CRISPR-Cas systems against invading nucleic acids.

Challenges and Limitations: The prevalence of CRISPR-Cas systems is concerning because while they may provide defence mechanisms, they also come with costs and limitations. For example, they are silenced by H-NS (Histon like Nucleoid Structuring, a DNA binding protein involved in organisation and compaction of the bacterial chromosome) in E. coli, and the risk of self-targeting leading to host cell death reduces their value as a defence system.

Moreover, analysis of acid mine drainage (acidic water resulting from mining activities and the impact on viral genomes in the ecosystem affected) reveals extreme CRISPR diversity driven by viral recombination which could render any spacer other than the most recent without a target. Recent experiments on CRISPR dynamics in Streptococcus thermophilus reveal that spacer sampling is not random, as there are a small number of dominant spacers with rapidly oscillating abundances. While bacteriophages can replicate in populations with one targeting spacer, they cannot in those with two.

Conclusion: In summary, this in-depth comprehension of the components and function of the CRISPR-Cas system reveals a remarkable immune system in bacteria and archaea that can selectively target and cleave foreign DNA. This knowledge has propelled the development of CRISPR-based genome editing technologies and holds immense potential for transformative applications in various fields, most notably, the exploration of new therapeutic strategies.

PART TWO: APPLICATIONS and ADVANCEMENTS- FUTURE of CRISPR

Discussion:

What scientists have learned by their discovery and study of the CRISPR-Cas mechanism developed by bacteria and archaea, they now aim to duplicate to build precise and targeted gene-editing tools for a wide range of applications in various organisms, including humans.

1.COVID applications

Introduction:

Since December 2019, the COVID-19 outbreak caused by SARS-CoV-2 has rapidly spread worldwide, resulting in overwhelming our healthcare systems and triggering global health concerns. Timely detection of infected individuals and effective therapy are crucial for pandemic control. Recent advancements in the CRISPR-Cas system offer promising opportunities for the development of innovative diagnostic and therapeutic approaches towards this objective.

Introduction to SARS-CoV-2:

SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus belonging to the β-Coronavirus subgenus of the Coronaviridae family in the order Nidovirales. It shares 79% similarity with SARS-CoV and 50% similarity with MERS-CoV. Among the seven known types of CoV that infect humans, SARS-CoV-2, along with SARS-CoV and MERS-CoV, causes severe respiratory symptoms ranging from mild to severe pneumonia and dyspnea, with potentially fatal outcomes. The genome of SARS-CoV-2 consists of a single RNA sequence approximately 30 kb in length, encoding sixteen non-structural proteins, four structural proteins, and nine accessory factors.

Pathogenesis of Sara-CoV-2:

During replication, the spike protein of the virus specifically binds to the host cell receptor ACE2, along with other host factors. These binding triggers cleavage of the spike protein by the cellular protease TMPRSS2, resulting in conformational changes necessary for virion fusion with cellular or endosome membranes and subsequent entry into human cells. Once inside the cytoplasm, the viral RNA is released and uncoated, leading to immediate translation of ORF1a and ORF1b, generating polyproteins pp1a and pp1ab. These polyproteins are further processed into sixteen individual non-structural proteins, which assemble into the viral replication and transcription complex. Replication and transcription of the genomic and sub-genomic RNAs occur within double-membrane vesicles derived from the endoplasmic reticulum, providing protection against cellular exonucleases and the innate immune system. The structural proteins (spike, envelope, membrane, and nucleocapsid) are translated at the endoplasmic reticulum and transported to the ER-to-Golgi intermediate compartment, where they assemble with genomic RNA to form virions. Virions are then released from the cell surface through exocytosis, seeking new host cells for infection. Each step of the SARS-CoV-2 life cycle involves interactions between viral and host proteins.

Researchers have focused on screening for essential host factors involved in the replication of multiple CoVs to develop broad-spectrum antiviral drugs.

SARS-CoV-2 is highly transmissible through direct contact with droplets or airborne particles from infected individuals, as well as indirect contact with contaminated surfaces. The virus can be transmitted even before symptoms appear, and the infectivity period ranges from 2 to 14 days. To combat the virus, effective diagnostics and treatments are crucial.

Diagnostic methods:

Diagnostic methods can be divided into two main groups: immunological assays and molecular assays. Immunological assays, like ELISA, detect viral antigens in respiratory secretions or antiviral antibodies in blood samples. Molecular assays, such as RT-qPCR, detect SARS-CoV-2 RNA in nasopharyngeal samples using TaqMan primers and probes for specific viral genes.

The qPCR assay is based on the principles of the polymerase chain reaction (PCR), a method that amplifies a specific DNA or RNA target sequence into millions of copies. It is a well-established technique for pathogen detection, gene expression analysis, and genetic testing.

Cas-based SARS-CoV-2 detection methods, such as FELUDA, DETECTR, and SHERLOCK, have been developed as alternatives to qPCR assays. CRISPR-based methods use Cas9, Cas12, and Cas13 nucleases to detect and differentiate SARS-CoV-2 variants. Customized crRNAs are designed to recognize and bind to target sequences, working together with the selected Cas nucleases. When the crRNA and Cas nuclease combinations encounter the intended target DNA, they trigger a signal response. Different combinations of CRISPR-based methods vary in specificity, sensitivity, and applicability. Cas12 and Cas13 have "multiple turnover trans-cleavage activity," allowing them to cut multiple target molecules without being inactivated. Cas9, on the other hand, can only perform "single-turnover cis-cleavage," cutting its target once and becoming inactive. This makes Cas12 and Cas13 more effective at generating signals.

Diagnostic advantages of CRISPR:

1. Simplicity and speed: CRISPR-based assays simplify the detection process by eliminating the need for complex thermal cycling and fluorescence detection methods required in qPCR. CRISPR-based systems use a simple colorimetric or lateral flow readout, allowing for rapid and easy interpretation of results.
2. Cost-effectiveness: CRISPR-based assays can be more cost-effective than qPCR assays since they do not require expensive thermal cyclers and fluorescent probes.
3. Specificity and sensitivity: CRISPR-Cas enzymes, such as Cas12 or Cas13, can be programmed to recognize specific target sequences within the SARS-CoV-2 genome. This programmability allows for precise detection and differentiation of viral strains and mutations, reducing the chances of false-positive or false-negative results.
4. Multiplexing capabilities: CRISPR-based assays can detect multiple targets simultaneously, known as multiplexing. This is particularly useful in the case of SARS-CoV-2, where the virus can mutate, and multiple variants may be circulating simultaneously.
5. Potential for point-of-care testing: The simplicity and speed of CRISPR-based assays lend them to be performed outside of traditional laboratory settings, enabling faster and more accessible testing in settings like clinics, airports, or remote areas.

CRISPR impact on viral pathogenesis:

Cas-crRNA complexes derived from CRISPR have demonstrated the ability to reduce viral loads in infected hamsters' lungs by degrading virus genomes and limiting viral replication in host cells. The CRISPR-based system has facilitated the creation of viral-host interaction screening platforms, enabling the identification of crucial cellular factors involved in pathogenesis. By utilizing CRISPR knockout and activation screening, essential pathways in the coronavirus life cycle have been revealed, including host cell entry receptors (ACE2, DPP4, and ANPEP), proteases responsible for spike activation and membrane fusion (CTSL and TMPRSS2), intracellular trafficking pathways for virus uncoating and budding, and membrane recruitment for viral replication. Furthermore, systematic data mining analysis has identified novel genes (SMARCA4, ARIDIA, and KDM6A) as potential pathogenic factors for severe Covid infection.

Therapeutic perspectives of CRISPR:

Although the CRISPR-Cas system promises to be a potential treatment for COVID-19, its off-target effects present a hurdle that must be overcome. This could be achieved by carefully designing the sgRNA and refining the specificity of the Cas protein.

sgRNA is a synthetic RNA molecule designed to guide the Cas9 enzyme to a specific target DNA sequence within a genome. It consists of two key components: CRISPR RNA (crRNA): This region of the sgRNA molecule contains a sequence that is complementary to the target DNA sequence. It serves as a recognition element to bind to the specific DNA region of interest.

Trans-activating CRISPR RNA (tracrRNA): This region of the sgRNA molecule is responsible for providing structural stability to the sgRNA and interacting with the Cas9 enzyme.

The sgRNA molecule is designed to be complementary to a specific target sequence in the genome that the researcher wants to modify. Once inside the cell, the sgRNA forms a complex with the Cas9 enzyme, which acts as a molecular pair of "scissors." The sgRNA molecule guides the Cas9 enzyme to the target DNA sequence, where it binds and creates a double-stranded DNA break at the desired location. This break stimulates the cell's natural DNA repair mechanisms, leading to the introduction of specific genetic modifications, such as gene deletions, insertions, or replacements. sgRNA is thus a critical component of the CRISPR-Cas9 gene editing system, allowing scientists to precisely target and modify specific regions of the genome in a wide range of organisms.

Another vital component of this therapy is delivery; getting the treatment into human bodies safely and efficiently is key. To date, research has largely been conducted on animal models, so further studies in humans are needed.

It takes a long time to develop vaccines and drugs, and the rapidly changing variants of SARS-CoV-2 may mean that existing ones are no longer effective. Despite advances in developing new vaccines, they still can't keep up with the mutation rate of the virus. CRISPR-Cas technology provides an advantage here due to its ability to use bioinformatics methods quickly to identify potential crRNAs for diagnostics and treatments. CRISPR-based screens combined with proteomics studies can also be used to understand viral replication and pathogenesis, as well as assist with choosing drug targets/candidates.

Conclusion:

CRISPR-Cas technology has changed gene editing, and provided important developments in screening, diagnostics, and treatments of various infectious agents, such as those related to SARS-CoV-2 and future emerging pathogens.

2. Sustainability applications:

CRISPR-Cas Genome Editing for Sustainable Bioeconomy: Addressing Food Loss, Waste, and Environmental Challenges

Introduction:

The global challenges of climate change, unsustainable food systems, food waste, energy crisis, and environmental degradation require innovative solutions for international development. Conventional technologies could be time-consuming, environmentally harmful, and cost ineffective. Therefore, biotechnological tools, particularly CRISPR-Cas genome editing, are crucial for enhancing food and energy resilience through eco-friendly bio-based products and circular bioeconomy principles aligned with Sustainable Development Goals (SDGs).

Methods:

The CRISPR-Cas system has the potential to improve bioeconomy by reducing food loss and waste throughout the food supply chain, valorising (increasing the value of) food, and plant waste through:

- CRISPR-Cas-mediated genome engineering of microbial cells for biofuel generation from low-cost substrates
- Effective CRISPR-assisted strategies for green bioplastics
- CRISPR-mediated editing in trees to enhance productivity and quality.
- CRISPR-assisted phytoremediation (targeting specific genes involved in pollutant uptake, transport, or metabolism to improve the plant's efficiency in removing contaminants from the environment).

Ethical implications:

Genome editing technology, referred to as a "New Plant Breeding Technology" (NPBT), has rapidly developed bioproducts from the laboratory and into global markets. There is an ever-increasing interest in research on genome editing techniques, which have created plant-based food supplies with market-oriented traits to reduce food waste and in some countries have released the first genome edited crops to the public. Since the 1990s, many countries have legislated the use of genetically modified organisms (GMOs) for beneficial reasons, such as food, feed, and processing. These GMOs include carrots, canola, Bt-cotton, Bt-potato, glyphosate-resistant soybean, and strawberry. Several nations--notably the United States, Brazil, Argentina, India, Canada, and China--have greatly increased their commercial production of GM food crops like soybeans, corn, and cotton. However, it remains a source of debate among consumers.

Role in crop enhancement:

CRISPR/Cas, Transcription Activator-Like Effector Nucleases (TALENs), and Zinc Finger Nucleases (ZFNs) represent a set of rapidly evolving genome editing technologies. By utilizing CRISPR/Cas9, researchers can accurately characterize genomic rearrangements and study the functions of plant genes. This enables enhancement of critical agronomic traits in field crops, such as yield, disease resistance, and nutritional content and is further accelerated by speed editing development. By targeting multiple genes simultaneously, the speed editing strategy aims to advance crop improvement and broaden the range of traits that can be modified. This seems to be the future of plant science.

Conclusion:

The CRISPR-Cas system offers a revolutionary DNA modification tool and because of the enormity of its potential, regulatory frameworks need to be addressed for the responsible and safe use of CRISPR-Cas edited products.

3. Cancer Research and Therapy Applications

Cancer Research

Introduction:

Cancer, a leading cause of disease-related death, has shown an increasing prevalence globally. Extensive research efforts have been directed towards the prevention and treatment of cancer, leading to significant advancements in various therapeutic approaches. Current cancer treatments involve surgery, radiotherapy, chemotherapy, and targeted drug therapy. However, these methods often face challenges such as incomplete tumour removal, damage to normal cells, and tumour drug resistance. In recent years, the emergence of gene therapy and gene editing technologies, particularly CRISPR/Cas9, has opened new avenues for cancer treatment. It is relevant to explore the applications of CRISPR/Cas9 in cancer research, including animal modelling, genetic analysis, gene therapy, and drug target screening.

Methods:

Cancer Modelling

1. Animal Cancer Modelling: Creating animal models with specific mutant genes has been shown to be a highly effective method for investigating gene mutations associated with cancer. Traditional methods of genetic modification in animal models are time-consuming and labour-intensive. However, with the advent of CRISPR/Cas9 technology, generating cancer models has become simpler. Researchers have successfully established tumour models in mice by using CRISPR/Cas9 to disrupt tumour suppressor genes. Zebrafish models have also been developed to study multi-gene interactions and understand the mechanisms of cancer occurrence.

2. Modelling Chromosomal Rearrangements: In cancer, chromosomal rearrangements play a significant role in cellular carcinogenesis. These

rearrangements result in changes in gene expression and contribute to the development of various cancers. Traditional methods of generating cancer models with chromosomal rearrangements involve complex genetic modifications. However, CRISPR/Cas9 technology offers a simpler approach by enabling targeted cleavage at specific rearrangement sites. This technology significantly reduces the time and cost required for modelling chromosomal rearrangements, providing an effective platform for studying cancer development and screening potential therapies.

3. High Throughput (large scale) Genetic Analysis of Tumour Cell Metastasis-Related Genes: The identification of key mutations that cause cancer is a challenging task. Tumorigenesis involves multiple genetic abnormalities acting together. High throughput screening (HTS) methods, characterized by their speed, sensitivity, and accuracy, have proven valuable in identifying these key mutations. Researchers have employed CRISPR/Cas9 library technology to conduct HTS experiments, enabling the identification of genes involved in cancer development and metastasis. This approach provides valuable insights into the mechanisms of cancer cell migration.

Conclusion:

CRISPR/Cas9 technology offers promising prospects for cancer research and therapy. It can be employed for targeted gene editing in cancer cells or for modifying immune cells to enhance their ability to combat cancer. Gene therapy using CRISPR/Cas9 has shown positive results in preclinical studies, indicating its potential for future clinical applications. Furthermore, CRISPR/Cas9 technology can be utilized to screen for cancer drug targets, facilitating the development of effective therapies. Although challenges remain, such as off-target effects and long-term safety concerns, ongoing research and improvements in gene editing technologies will undoubtedly revolutionize the treatment of hereditary diseases and cancer.

Chemotherapy Resistance

Introduction:

In the face of new targeted cancer drugs, curing metastatic solid tumours is still elusive due to chemotherapeutic resistance. While there is an understanding of some mechanisms behind this resistance, much more research is needed to understand how cancer cells defeat treatments that have proven to be effective. The orthodox approach of taking resistant clones from a lab setting and testing out their drug-resistant traits in a clinical context takes too much time and doesn't equate with real life scenarios.

Methods:

CRISPR technology holds great promise for generating libraries of cancer cells carrying single guide RNAs (sgRNAs) to uncover novel mechanisms of resistance. CRISPR knockout screens involve systematically disrupting specific genes to evaluate their impact on resistance mechanisms. Conversely, CRISPR activation screens facilitate the overexpression of target genes to investigate their role in resistance. CRISPR inhibition screens enable the suppression of specific gene functions. By employing a combination of knockout, activation, and inhibition screens to study multiple genes simultaneously, researchers can uncover both gain-of-function and loss-of-function mechanisms underlying resistance. Synthetic lethality is a process that occurs when the simultaneous disruption of two or more genes leads to cell death, providing a therapeutic opportunity. CRISPR technology can be leveraged to identify gene pairs or networks that exhibit synthetic lethality. Specialized approaches enable the identification of multiple genes that may contribute to resistance, allowing for a more comprehensive understanding of the molecular landscape underlying treatment failure.

Limitations:

Off-target effects, incomplete knockout or activation, and clonal heterogeneity (presence of genetically distinct subpopulations of cells within a tumour) within cell populations are important factors that need to be considered when interpreting CRISPR-based experimental results. Careful experimental design, validation, and data analysis are crucial to ensure the reliability and reproducibility of findings.

4. Antibiotic Resistance Applications

Introduction:

The antibiotic resistance crisis is a major threat to global healthcare, drawing considerable attention worldwide. ESKAPE bacteria are identified as the most susceptible to antibiotic resistance, causing both community- and hospital-acquired diseases. Antibiotic resistance can be caused by intrinsic, acquired, or adaptive mechanisms. Intrinsic resistance involves bacteria reducing the concentration of antibiotics, while acquired resistance occurs through gene expression changes and biofilm formation. Resistance can also spread through mobile genetic elements (MGEs) and horizontal gene transfer (HGT). The need for research and development (R&D) of novel therapeutic compounds to combat bacterial infections and antibiotic resistance is important. One of the challenges in introducing new antibiotics is the high cost associated with research and development. Antibiotic therapy can also affect the human symbiotic microbiome, and a portion of the pathogen population often survives due to drug tolerance or persistence. Increasing the concentration and duration of antibacterial agents may lead to side effects and toxicity, limiting the potential specificity of innovative drugs.

Methods:

Scientists can engineer the CRISPR-Cas system to produce a special CRISPR RNA (the transcription or copy of the bacterial genetic material that contains information stored sequentially of the antibiotics/viruses they have encountered before) that specifically targets genes associated with antibiotic resistance. They design the crRNA to match the sequences of these resistance genes. Once inside the bacterial cell, Cas proteins team up with the crRNA to search for matching sequences in the bacterial genome. The crRNA guides the Cas proteins like a map, leading them to the antibiotic resistance gene. When the Cas proteins find a match between the crRNA and a resistance gene, they snip the bacterial genome at the exact spot where the resistance gene is located. Now that the resistance gene has been cut, the bacterial cell tries to repair the damage. However, repairs can sometimes introduce errors, leading to mutations or rendering the resistance gene non-functional. With the resistance gene damaged or mutated, the bacterium loses its ability to resist antibiotics. Resistance plasmids, which are genetic elements that can transfer antibiotic resistance between bacteria have also been targeted using the system making bacteria more susceptible to antibiotics. Studies have demonstrated the successful removal of plasmids conferring resistance to methicillin, colistin, and carbapenems.

Mechanism of delivery:

Scientists have been using different methods to deliver CRISPR, including viruses and non-viral approaches.

1.<u>Viral delivery</u> is a popular method, and one type of virus called temperate phage has been studied. These bacteriophages can carry the CRISPR genes and inject them into bacteria when they infect them. However, there are two main issues with using them for delivery. First, is the limited range of bacteria they can infect. Second, they are only effective against bacteria on the surface and outside of cells. To overcome these challenges, researchers have been trying to expand the range of bacteriophages and improve their delivery. They have explored modifying the phage's structure to infect a wider range of bacteria.

2.Additionally, they have used chemical substances to help them enter bacterial cells, remain active, and avoid the immune system.

3.Another delivery method being explored is using liposomes, which are tiny bubbles made of fat. Liposomes have been used to deliver drugs because they are stable and can target specific areas. Researchers have also used cationic liposomes to deliver bacteriophages, which helped protect them from antibodies and remove bacterial biofilms. The term "cationic" refers to the positive charge present on the surface of the liposomes. This positive charge is achieved by incorporating cationic lipids into the lipid bilayer, which is the structure of the liposome. Cationic lipids contain positively charged molecules, such as quaternary ammonium compounds, which give the liposome its positive charge. The positive charge of cationic liposomes allows them to interact with DNA or RNA, which are negatively charged due to their phosphate backbone. This interaction between the cationic liposomes and the genetic material allows the liposomes to package and protect the genetic material, helping to deliver it to target cells.

Challenges:

Biofilms are communities of bacteria that stick together. They form a protective layer that can make it difficult for CRISPR to work properly. One way to overcome this is to use other bacteria that are already in the biofilm to help deliver the CRISPR tools more effectively. These bacteria, called donor bacteria, can carry the CRISPR components and deliver them to the target bacteria in the biofilm. Additional research is needed to ensure safe and effective delivery, especially in clinical settings.

Conclusion:

The use of CRISPR-Cas systems offers promising strategies to combat antibiotic resistance by specifically targeting and eliminating resistance genes in bacteria.

CRISPR Conclusions:

The CRISPR-Cas9 system has revolutionized the field of biomedical science by offering precise and efficient genome editing capabilities. Its development was recognized with the 2020 Nobel Prize in Chemistry for its founding scientists, Doudna and Charpentier. While there are still limitations to the technology, ongoing research is underway to address accuracy and insertional mutagenesis and the possibilities of base editing and prime editing platforms offer scope to make further breakthroughs. Ultimately, genome editing using CRISPR-Cas9 technologies provides a potential solution with cell repair and axonal regeneration for ocular disorders, inherited retinal diseases, corneal defects, optic nerve injuries. The eye is an advantageous target for gene therapy due to its accessibility, immune privilege, and distance from other organs. This concluding application for the gateway to the most fundamental human sense of vision also carries personal significance, filling me with hope and happiness. All CRISPR achievements, even if nascent, highlight the best blessings of science and innovation, offering the potential to transform public health.

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