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CRISPER-Cas 9: A Genome Engineering Tool

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CRISPR is an RNA guided genome editing technique of genetic engineering which works like genetic scissors. Based on simplified version of bacterial CRISPR-Cas9 antiviral defence system. It is more accurate, faster and cost efficient than other genome editing methods. There are two components in this system: First component includes a single guide RNA (sgRNA) of system which will identify target sequence in genome and Second component will include Cas9 nuclease of system which will act as a pair of scissors to spilt the double strands of DNA. CRISPR has promising therapeutic applications.

Introduction

CRISPR's first description was given by Ishino in year 1987. CRISPR which is also known as clustered regularly interspaced short palindromic repeats. For protection of bacteria and archea against encroaching nucleic acids of plasmids and phages CRISPR and CRISPR – Cas are a part of the acquired immune system of bacteria and archae. Cas9 is more widely used type because its faster, cheaper and more accurate than other genome editing methods. To cleave the DNA at certain sites Cas use single guide (sgRNA) to form complimentary base pairs with the targeted DNA. Cas9/sgRNA is a two component system which is very accurate in gene editing. In this system the identification of specific sequence in genome is done by sgRNA and the DNA sequence will be cleaved with the help of Cas9 protein which will act like a pair of scissors.

There are two factors that decides its specificity:

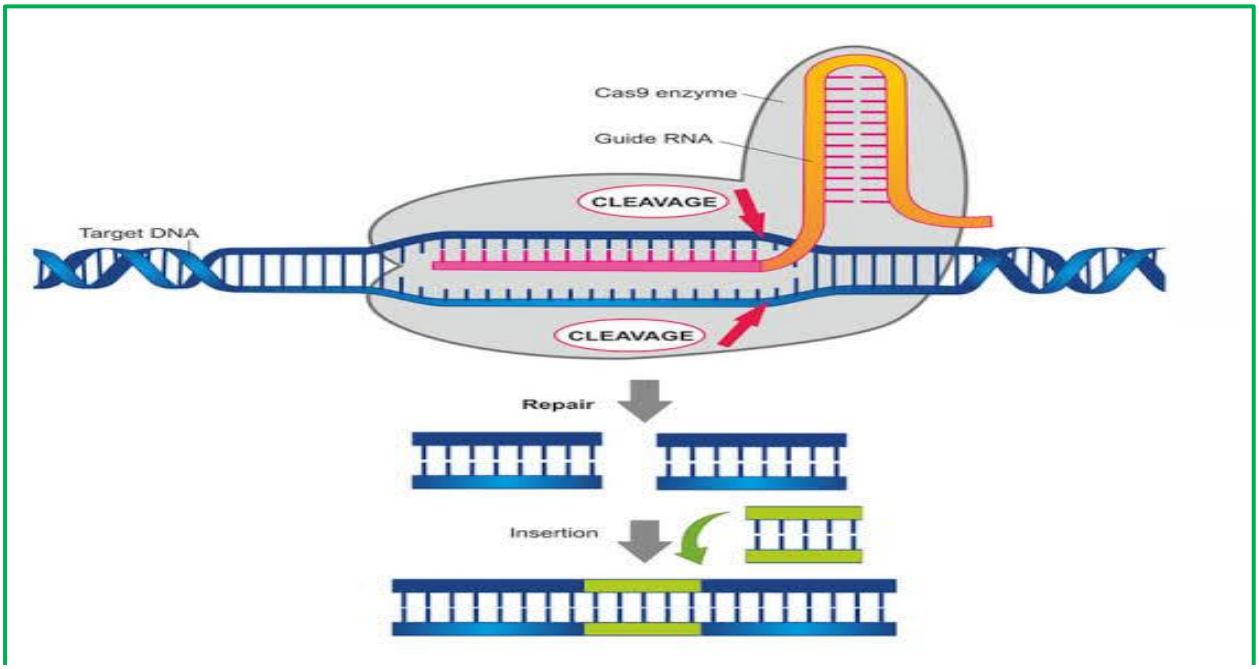
1. Target Sequence
2. PAM Sequence

Target Sequence has a length of 20 bases as it's a part of each CRISPR locus in the crRNA array. Cas9 identifies the PAM sequence present on the host's genome. To find the correct sequence in host cell's DNA Cas9 protein will take assistance of crRNA. After which it depends upon the Cas9 variant either to make a single or double stranded break at the correct location.

Mechanism

Genome editing system occurs naturally in bacteria from which CRISPR–Cas9 is adapted. Cas9 nuclease is the main component of CRISPR which has two catalytic active sites RuvC and HNH, and single guide RNA (sgRNA) derived from the crRNA and trans acting CRISPRRNA. sgRNA directs the Cas9 target site with pairing of base in presence of PAM on the opposite strand sgRNA , which results in site specific DNA double strand breaks that are later repaired by HDR(homologous directed repair) if the homologous sequences are not available then they could be repaired by NHEJ (non homologous end joining). There is precision in gene correction or replacement when HDR are used compared to NHEJ which

can induce small insert or delete (indel) mutations. By the inactivation of either RuvC or HNH Cas9 can be reprogrammed into nickase (nCas9) and by inactivation of RuvC and HCH it can be reprogrammed into catalytically inactive Cas9(dcas9). When S.pyogenes Cas9 taken for structural analysis it was revealed that additional insight are present into mechanism of CRISPR. The NHEJ pathway is highly effective than HDR pathway as Gene Knock out is usually more effective than the Gene Knock in. Crispr is new promising therapy of genetic disorders in which direct editing of disease related mutations is possible. Lots of efforts are given to improve the specificity, efficacy of gene editing and efficiency of delivery in CRISPR.



Different approaches to edit genes using CRISPR-Cas9

There are various approaches which are used when one has to edit genes. In case of CRISPR – Cas9 there are three main approaches that are used to edit genes. In the first approach multiple transfections in different components can be avoided by using plasmid based CRISPR – Cas9 system which will encode Cas9 proteins and sgRNA from the same vector. Mixture of Cas9 mRNA and sgRNA is delivered as second approach. Mixture of Cas9 protein and sgRNA is delivered as third approach. Greater stability is seen in plasmid based CRISPR – Cas9 system approach compared to the approach that uses sgRNA mixture with Cas9 mRNA. Cas9 mRNA mixture with sgRNA will be directly delivered to the target cells which expresses the Cas9 protein and will form the Cas9/sgRNA complex inside the cells after which it will edit the gene. One of the advantages is that the duration of gene editing is fixed. For mRNAs to exert their effects have to enter cytoplasm. Lower off targets are seen in the delivery of mRNAs compared to the plasmid based approach. Lower levels of cytotoxicity are seen in primary cell and cell lines . When mRNA encoding Cas9 protein is used. Ribonucleoprotein complexes (RNPs) have some advantages on its direct delivery such as rapid action; high gene editing efficacy; reduced off target effects; reduced toxicity and many more. When a purified Cas9 protein is positively charged and forms complex with sgRNA Cas9/sgRNA ribonucleoprotein complexes (RNPs) are formed (Desai *et al*, 2020)

Applications and Future Prospects

Some of the applications including the therapeutic ones are still under development. The information in these applications are the proven potentials of CRISPR gene editing and also

shows what it's capable to do so take a note of that. Derived from the inner cell mass (ICM) of blastocytes embryonic stem cells are Pluripotent stem cells (PSC) and hence shows difficulty in genome editing. CRISPR - Cas system can efficiently control the genome without complex process. This accelerates the stem cell study. Reversible and efficient genome editing can be enabled by combining CRISPR - Cas system and reverse system for comprehensive gene analysis. Simplification of knock in, knock out, transgene and reversible genome engineering which has been achieved in stem cells is done by CRISPR - Cas system. There is a acceleration seen in the generation of stem cell lines specially in disease models because of genome editing with CRISPR - Cas system. The cause of neurogenerative diseases are neuronal impairments, stem cells models shows appropriate insights in study and cure of neurogenerative disease. Recent study shows that modelling of clinical pathogenic mutations can summarize the specificity of neurogenerative disease for example amyloid β (Aβ)'s generation in Alzheimer's disease (AD). Validity and safety before clinical applications are lightened up by direct gene correction with CRISPR - Cas system. Researchers have used Cas9 to inactive a endogenous retrovirus in pigs and also to engineer T cells as a start to develop advanced immunotherapies to target cancers.

Merits

Over conventional protein guided genome editing tools such as ZFN and TALEN, CRISPR – Cas9 RNA guided genome editing gives many advantages. ZFN or TALEN based tools requires de novo synthesis which is way complex than CRISPR – Cas9 which only requires design of complementary sgRNA to target a new site. With the multiple sgRNAs that can target different genomic loci, CRISPR – Cas9 has capability to edit these genomic loci in parallel this property is called multiplexing. TALEN also has some advantages over CRISPR-Cas9. CRISPR – Cas9 has restricted targets because of presence of PAM sequence and a guanine at the 5′end 11, when compared to TALEN targets in presence of thymine at the 5′end is the only restriction. Which means CRISPR – Cas9 has less target genome sites than TALEN.

Conclusion

CRISPR-Cas9, the RNA guided genome tool has many advantages over protein guided counterparts. It has various therapeutic benefits but before it is used for patients benefit there are some challenges that needs to be overcome. As there are rapid advancements happening in CRISPR-Cas9 technology we can still believe that in future it can revolutionize gene therapy research and become the convenient tool for human gene therapy.

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