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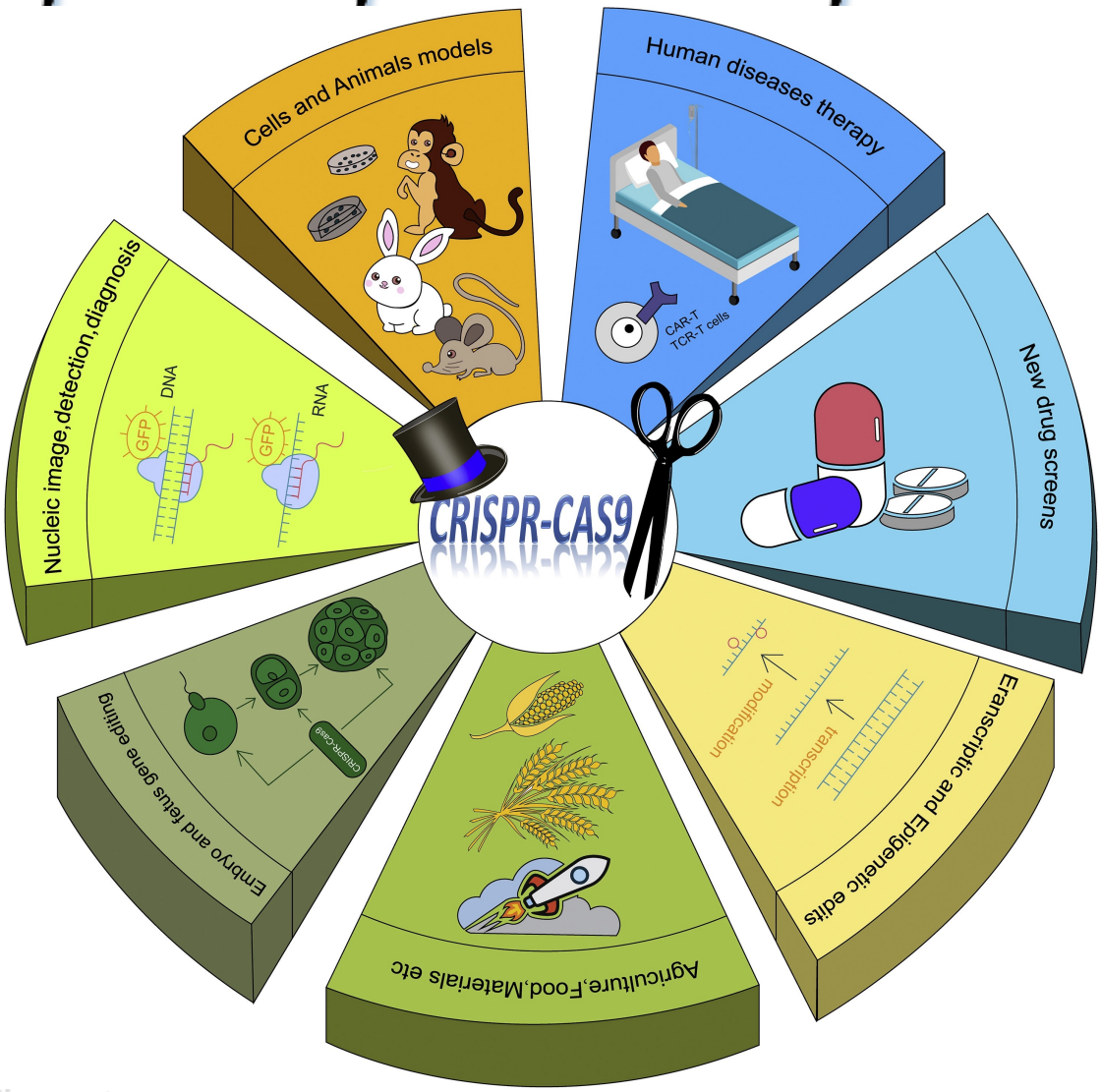
The Biochemistry Chronicles



THERAPEAUTIC APPROACH BY A MODIFIED CRISPR-CAS SYSTEM

Background:

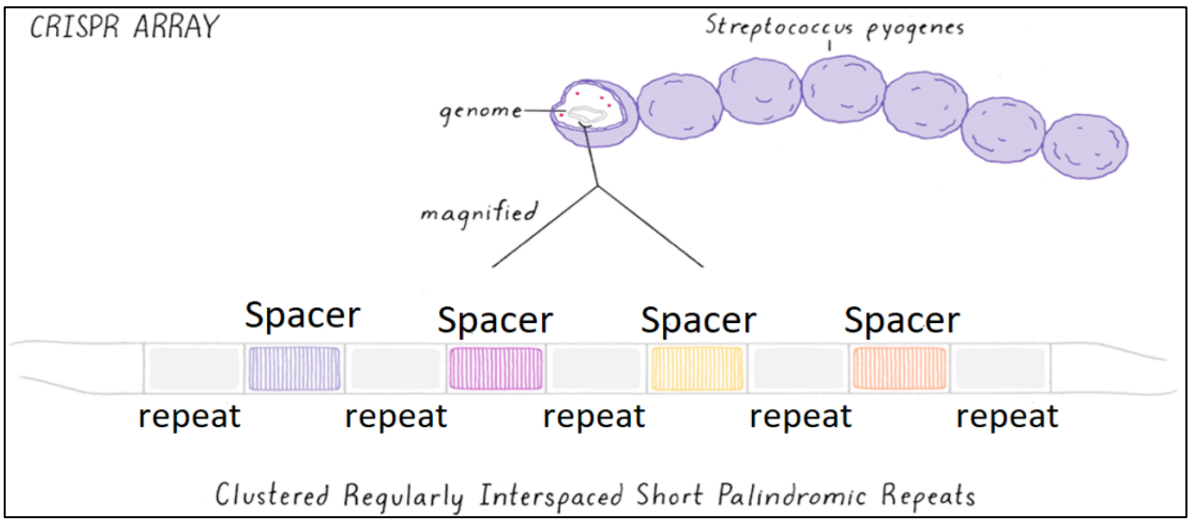
Since the discovery of biological function of restriction endonuclease and recombinant DNA technology in early 1960s, to the invention of polymerase chain reaction (PCR) in 1985 and the demonstration of CRISPR mediated genome editing in 2012, each new breakthrough discovery in biotechnology further improved our ability to manipulate DNA. In particular CRISPR-CAS system or 'genetic scissors' has become the most effective, efficient and accurate method of genome editing tool in all living cells. Now it becomes a new era in molecular biology from treating and curing diseases to agriculture sector also.

CRISPR Cas-9:

Clustered Regularly Interspaced Short Palindromic Repeat refers to the unique organization of short partially repeated DNA sequence regularly interspaced by spacer DNA (short fragment of viral DNA serve as genomic memories). CRISPR and its associated protein (CAS 9) is a method of adaptive immune system in prokaryotes to defend themselves against virus or bacteriophage[1].

Guide RNA (gRNA) and CRISPR associated protein (Cas-9) are two essential components in this system. Cas-9 is large multidomain enzyme having helicase, nuclease, polymerase and polynucleotide binding activity. Cas-9 consists of two regions – Recognition (REC) and nuclease(NUC) lobe[2].

Nuclease lobe (NUC) again composed of RuvC, HNH and Protospacer Adjacent Motif (PAM) interacting domains. PAM sequence is a short, conserved DNA sequence downstream to cut site. RuvC and HNH domains are used to cut each single stranded DNA, while PAM interacting domain confers PAM specificity and is responsible for initiating binding to target DNA. Recognition lobe (REC) is responsible for binding the guide RNA[3]. Guide RNA is made up of two parts – CRISPR RNA (crRNA) and transactivating CRISPR RNA (tracrRNA). The crRNA is 18–20 bp in length that specifies the target DNA by pairing with target sequences, whereas tracrRNA is a long stretch of loopserve as binding scaffold for Cas-9 nuclease.



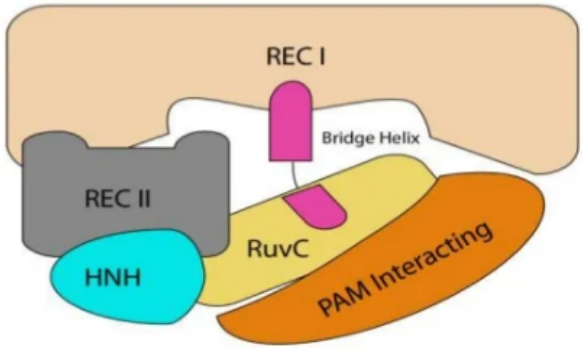


Fig: Schematic diagram of Cas-9 Protein

three steps – Recognition, Cleavage and Repair. The designed is sgRNA (synthetically designed by combining crRNA and tracr RNA) directs Cas-9 and recognizes the target sequences through its 5' complementary crRNA base pair component. Then the CAS 9 nuclease makes double strand breaks (DSB) at a site 3 bp upstream to PAM. Finally, the DSBs are repaired by host cellular machinery[4].

immunogenicity and non-integration into the host cell genome compared to other viral vectors.

Modified CRISPR Cas Sys -tem:

A cell retains its DNA for life and passes an identical copy to daughter cells, so any changes in DNA are permanent; whereas RNA is a more transient molecule, transcribed from DNA and degraded shortly. As change in DNA is permanent, it especially required when comes to treating an inherited genetic disease. But for an infection, an injury or some other temporary disease, being able to temporarily modify a gene through RNA targeting makes more sense.

Previously the only enzyme that could target RNA was Cas-13, but that had a messy side effect; when it recognized a particular gene the enzyme began cutting up all the RNA around it. This property makes Cas-13 effective for diagnostic tests

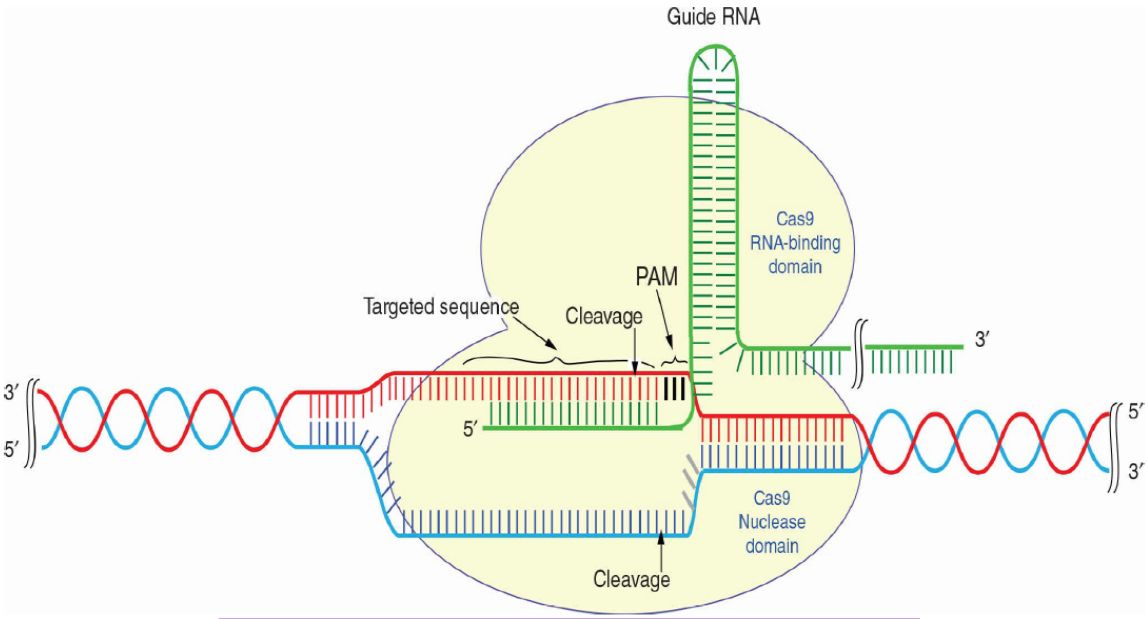


Fig: Overview of the mechanism of CRISPR-Cas9

For safe and effective delivery of components into the cell, currently 3 methods are used – physical, chemical and viral vectors. Among them adeno-associated virus (AAVs) are the most commonly used vectors due to their low

where it is used to detect the presence of RNA; but not very useful for therapeutic purposes where target cuts are required. Here comes Cas-7-11 that has unique characteristics of more precise form of RNA editing, analogues to the CAS-9

enzyme for DNA. However, the massive Cas-7-11 protein was too big to fit inside a single viral vector[5]. Cas-7-11 was like an amalgamation of five separate Cas enzyme, fused into one single gene. To determine the overall structure of Cas-7-11, Cryo-electron microscopy was used. The structure of Cas-7-11 revealed how pieces assembled and which parts of the protein were critical to recognizing and cutting RNA. The structure also illuminated section of protein that was not serving any apparent functional role. These findings suggested that Cas 7-11 can be re-engineered by removing those pieces to make it small enough without taking away its ability to target RNA. This new compacted version is known as Cas-7-11S. With Cas-7-11S packaged inside a single viral vector, the system was delivered into human cells and efficiently targeted RNA.

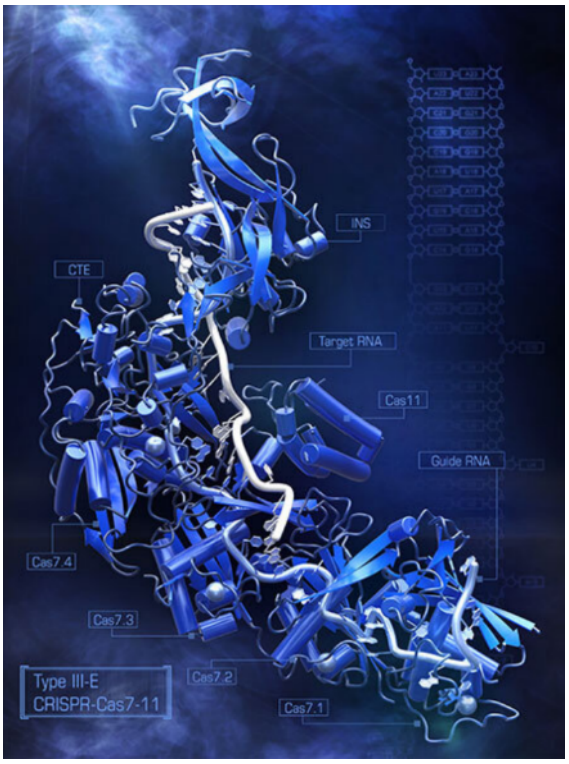


Fig: Cas 7-11 protein

An era is about to start in gene therapy which is RNA directed and modification is also under stringent control by modified CRISPR Cas system.

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AMBI NEWS

The AMBICON 2022 conference held in Bangalore had been great combination of academics, sharing ideas, catching up with people of our fraternity and enjoyment.

The representation of our state chapter was at its zenith this time involving paper presentations by Dr. Sanchayan sinha, Dr. Satwika sinha , and Dr. Priyanka datta.

There were invited lectures by Dr. Soma Gupta, Dr. Biswajit Saha from our state.

Dr Priyanka is selected as our new state representative in AMBI. This time the Zonal Representation of Eastern India involving states of Assam, Bihar, West Bengal, Odisha, Arunachal Pradesh, Manipur, Tripura, Meghalaya, Mizoram came to our state and Dr Soma Gupta is chosen to represent the post with full dignity.

We are proud of all the members who have attended and represented our state this time and will encourage other members to do so in upcoming days. Together we will work and make our state chapter activities the best of all in our own way. We might encounter constraints and challenges, we will try to convert them into newer opportunities.

Guidance from seniors and helping hand from juniors remain as our main strength to work and keep ourselves motivated in due course of time. With this in mind we are moving towards our next meeting – Annual State Chapter Conference of West Bengal (**AMBICON WB 2022**) scheduled to be organised on 8th and 9th September 2022 at Novotel, Kolkata. The conference is preceded by a one day preconference CME – **Immunoassay Conclave**, organised by Dept. of Biochemistry, IPGME&R and SSKM Hospital, Kolkata on 2nd September 2022. AMBI WB is looking forward to host all the members on these upcoming academic feasts.