# AN OVERVIEW ON CRISPR'S FUNCTIONAL PRINCIPLES AND IT'S ASSORTED APPLICATIONS

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**Abstract**

CRISPR-Cas9, the clustered regularly interspaced short palindromic repeat phenomenon associated with cas9 protein, currently accorded with noble laureate accreditation has been referred to as the most versatile and revolutionary genome-editing tool in accordance to its credibility and efficiency by allowing selective perturbation of an individual's genetic elements and precisely the reverse engineering of mutated genome, responsible for unintended and incurable alterations, surpassing the previously employed tools like zinc finger nucleases (ZFNs) or transcription activator-like effector nucleases (TALENs) in terms of intuitive work profile and adaptable response. This system is employed by bacteria and archaea to foster adaptive immunity and perpetuate everlasting memory against viral pathogens. Initial acquisition begins withprotospacer recognition followed by the involvement of tracrRNA associated with CRISPR RNA duplex and finalized by RNA guided DNA cleavage. Furthermore DNA modification and modulating complex transcriptive arrayshas been made facile. While numerating the aptness, it's scattered across genome editing, eradicating diseases, economical drug development, biotechnology and empirical agricultural advancement.

**Keywords:**CRISPR, genome editing, bacteria.

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## 1. Introduction

Since the discovery of DNA double helix model, a fervid approach of making double stranded breaks and hence revolutionizing the genome editing field with site specific modification in DNA was considered insurmountable but in the past decade, this immunological cascade has enkindled genome editing revolution which was previously perplexed with rather unfeasible methodologies based on protein recognition ,namely zinc finger nucleases (ZFNs) and TAL effector nucleases (TALENs)which was perhaps a quixotic procedure in terms of design and formation of proteins. [1] Contrarily CRISPR, the clustered regularly interspaced short palindromic repeat sequences which are resorted against lethal bacteriophages and viral encroachers by prokaryotes and archaea thus designated as the primary defense strategy employed by these primates for safeguarding the host is found to be astounding and noteworthy in terms of fostering an inheritable change in the double helix structure.[2]

This system is categorized into two parent classes, one and two which are further bisected into six classes. Class I is mainly observed in prokaryotes which covers for 90% of primates whereas remaining one tenth reside with class II system which comprises of ribo-nucleoprotein assembly. [4] As referred by remarkable researchers, CRISPR Cas helps in pinpointing target molecules which back up new drug development. It's knock out ability gives assistance for wantonly silencing or triggering the unknown gene which therefore accords for the documentation of it's functionality and importance called knock out screening. This technique also accounts for advancement in animal models impersonating intricate diseases and also provides detailed knowledge of cell signaling cascade and it's interrelations, that aids in cellular communication. All these perks act as guiding forefront for precision medication and personalized drug therapyalong side with error free editing.[3]

## 2. Mechanism of CRISPR –

As discussed above, that wide array of bacteria and archaea possess this sophisticated defense approach of crispr proteins under the aegis of guide RNA against bacteriophages and plasmid transfer.

### 2.1. Following steps are implied for the same

2.1.1. Once the invader targets the cells, specified genetic elements from the intruder are fragmentized into minuscule pieces of DNA which are then inserted into CRISPR spacer array within the host bacteria.

2.1.2. In addition, it gets embedded into the host chromosome and keeps the genetic record of preceding infections, furthermore facilitating efficient prophylaxis. [1]

2.1.3. The system produces intermeddling via crRNA after locating protospacer adjacent motif, abbreviated as PAM sequence.[5]

2.1.4. Alteration and redevelopment of guide sequence prepared in relation to target DNA binds to the complementary target strand and produces changes, thus editing the genome.[6] Most prominent and versatile type used in subtype II-A which uses Sp Cas 9 protein of class Streptococcus pyogenes. [7]

It follows three crucial phenomenon in chronological order i.e. adaptation, maturation and interference for which specific coded proteins are required.

Adaptation stage corresponds to correct sequence recognition of invader species and amalgamating CRISPR array which is facilitated by establishing the correct position of PAM that is responsible for correct recognisition of self and non self genomic sequence.[8]

Furthermore, in maturation stage the incorporated complex does transcription of the RNA molecule which is denoted as pre-crispr RNA {precr RNA}which is categorized successively into smaller sequences consisting of spacer molecules and required set of nucleotides.[9]

Moving forward to final stage, interference is completed with the assistance of cr RNA effector protein complex which exhibits the activity of "Molecular scissors" guided by RNA.

Cas 12a variant has multiple re-check mechanisms for precision tracking of focused sequence.[10] Domains involved in pinpointing the target sequence are WED II-III and REC I which after locating the sequence of interest, gets incorporated in PAM duplex via loop-lysine helix loop domain made up of amino acid lysine and prolines which assist the unwinding of helical strand.[11]This process enables the crRNA PAM complex to do its job. After the introduction of double stranded breaks i.e. accompanied by alterations at molecular level in PI domain caving it's path to site of action.The activity is kept under supervision via REC linker, the "lid" and "REC finger".[12,13] Polarity and orientation also influence the endonuclease activity so that proper cleavage takes place at distal end.Then the arrangement segregates itself. Furthermore,the cleaved strand is subjected to repair mechanisms via, Homologous pairing or non homologous end joining.[14]

Sequential pairing is done complementary to double stranded break which corrects the altered sequence. Cas 12a produces a cut near adjacent base pairs whereas Cas9 makes a blunt cut in double stranded DNA and demands the presence of PAM and crRNA alongside with the tracrRNA whereas 12a variant only required cr RNA so its preferred over Cas9 as it also uses just a singular vector.[15] It also offers sequence selectivity and downstream splicing which ensures errorless end joining also hampering the occurrence of undesirable variations.

Another discovered type is Cas13 whichis a type of RNA endonuclease that acts solitarily on ss ribonucleic acid and is distinguished on the basis of its capability of being attached to the Watson and crick helical arrangements even after unwinding, this phenomenon is termed as "Collateral damage".[16,17]These exceptional arrays are found in 28-37 base pairs with varying symmetrical arrangements, undergoing conformational changes to form hairpin like circular structures.[18] Till date 19 subtypes have been recorded which have been scattered over six classes.[19]

### 2.2. Sequential order of adaptive immune response aided by CRISPR includes

Firstly, cognition analysis is done by Cas1 and 2 in response to invasion. Followed by, fixation of protospacer with target nucleotide arrangement.The primary sequence of CRISPR array is nicked specifically to produce crRNA and their advancement leads to formation of interference complex which helps inactivating the phage DNA.

Both Cas 1 and 2 are crucial in acknowledging the spacer array as, in-vitro studies concluded their absence rendered the reorganization non-viable. Cas 1 proteins are heterogeneous protein arrangements that correspond to nucleases and integrases, which attach to Watson and Crick helical arrangement in a precise manner.[20,21] Characteristic Cas2 protein have either RNA or DNA like arrangement, comprising dimer bridges between Cas1 and 2 domains which attaches to phagic DNA and aid their incorporation into spacer array respectively.[22,23]

If bacteria is further invaded, its viral sequence information is added subsequently in successive order.[24] The CRISPR complex assists protospacer assembling and crRNA is later established complementary to CRISPR sequence. There are multiple spacers for a particular phage.[25] This is followed by transcription of tracr ribonucleotide arrangement where complementary base pairing results in formation of double stranded complementary structure which is a potential target of interest for RNAase III to synthesize CRISPR RNA, which binds with Cas amino acid sequence that crRNA which identifies invader viral particles.[25,26,27] Interference is commenced by recognition of PAM sequence which is subjected to annealing and intricate signaling via protospacer, causing structural changes in cascade that initiates DNA disruption.[28]Cas 9 employs the use of both CRISPR and tracr ribonucleotide arrangement for viral DNA degradation using RNaseH / Ruv c molecular scissors. The favored target of action for type III systems in messenger RNA.[29] But they can also disrupt DNA genome using Cas10 amino acid sequence.[30]Various studies and research programmes deemed CRISPR as a part of Lamarck's evolution as genomic alterations are passed on to successive generations.[31] This statement is contraindicated by studies

proposing it as Darwin's form of evolution.[31,32]

**Applications**
It's used as a versatile gene editing tool employed for food as well as agricultural advancements, e.g. it's been employed to formulate enhanced immunity eatables namely probiotics in yogurt and modified crops. [33] Furthermore, its usage is deployed in knock out technology or silencing a gene in humans and microbes e.g. Candida albicans has been subjected to same. [34]

It was recently integrated in female anopheles genome to cut down malarial transmission. [35] It's efficacy was tested on a human patient suffering from sickle cell anemia. [36]In vivo testing on mice was done in 2020, with active HIV strains and promising results were obtained with 60-80% of viral DNA dissolution and removal. [37]Recently in march 2020,same technology was employed in ophthalmic ailment treatment referred to as lebel congenital amarurosis. [38]It also has the potential of reproducing new species as well as recovering the extinct ones.[39]
The same strategic phenomenon was also employed as a diagnostic toolfor molecular testing with specific nucleic acid identification of sequence of interest and to check critical molecular sensitivity. [40,41]By combining it with various substrates, a particular sequence or compound of interest can be detected e.g. SPRINT technology which stands for SHERLOCK based profiling of in vitro transcription used to detect metabolic remnants in patients.[42]

An expectable and formularized control over zygosity can be attained with this noble approach. With this particular gene tech, pluripotent stem cells were synthesized.[43] A giant leap of faith was taken in case of stem cell research which turned out to be highly beneficial for people suffering from beta thalassemia for which, stem cell progenitor level replacement was considered as therapeutically effective, by altering mutation in HBB gene. [44]

It has also assisted researchers in gaining progressive knowledge about etiological and therapeutic alternatives for Alzheimer's disease based on genes involved for the same, especially of amyloid type. CRISPR is also an unmatchable tool for screening and target revelation on a genetic basis. [43] It's role in cancer treatment is highly appreciated as it targets all the potential objectives which can be possibly manipulated i.e., gene editing of mutated sequences and finding desirable aims to be focused for treatment. [44] One such example is screening of oncogenes and related cells that regulate the cytology of cancerous cells and finding possible targets viable for therapeutic manipulation. It's also employed for the study of various supporting factors that facilitate or trigger cancerous side of normal cells.[43] This approach also aides in constructing disease models especially to gain insights in oncological mediated drug discovery and evaluate occurrence of resistance and causative factors. It's done in case of SP3B1 which was experimentally identified as a potential therapeutic target via tagging of genomic sequence and alleles to differentiate their functionality.Precisely imitated organ models depicting human intestine was constructed via CRISPR to study cancerous growth and also genes culprit for oncological growth in colon and rectum were taken in consideration mainly APC, TP53, KRAS, SMAD4, PIK3CA. [45]

New and relevant connections between previously discovered biological pathways and their interactive nature can also be undertaken from this technology. It gives worthy information and allows us to comprehend numerous therapeutic approaches yet to be maneuvered.[43] Genetic alterations by using this approach were utilized in mammalian cells focusing on DYRK1A and GRIN2B sequence with 68% precision. Singular deletions or removal of one or two genes can also be done and the same was exhibited by the extirpation of EMXI gene.[46]

Customized singular guide RNAi libraries can be used for research of a specific gene or a wider genetic region of interest.This noble approach can be extensively applied for genetic upgrade and transformation by removing or adding a gene.[47] Gene silencing helps us to gain insights on function and importance of a given gene in a genetic sequence. By stopping it's activity and studying underlying mechanisms affected by the same. Complementary to this concept is

gene knock in where instead of silencing/ removal, you insert a genomic sequence externally for research purposes.[44]

The influence of non-messenger RNA's can be also be studied. Clustered regularly interspaced short palindromic repeat interference, also known as CRISPRi helps in hampering and demolishing a gene function by showing increased inclination towards the selected target referred to as gene silencing.[47]

Altering nuclease domains forms dCas 9 i.e. referred to as nuclease dead Cas protein which binds to DNA sequence without inducing any cuts. It can also terminate the gene if anchored at initial point by adhering and further disrupting the transcription assembly. This effect can be multiplied by the use of KRAB domain also known as Krupper associated box which addresses chromatin factors for influential silencing which is reversible in nature.[47]

Their study revealed that every given gene has a specified role in precluding cellular requirement of a particular factor responsible in it's growth. Moreover, these genetic expressions govern the complexity and severity of tumor growth.[45]

Applications of CRISPR technology is seen in finding alternatives of peptide – mimetic drugs. By doing gene editing of peptide transporter and the discovery of Q10 as a site of action for COQ2 mutated allele correction.[44]

Dosage relevant control of FOXG1 disease in terms of FOXG1 syndrome was also studied extensively. In patients suffering from severe genetic condition due to change in COL7A1 gene referred to as recessive dystrophic epidermolysis bullosa, regularity in collagen was successfully achieved. In terms of Duchenne muscular dystrophy arising from skeletal muscles exogenous addition in DMD exon corrected the change, producing promising effects.

Furthermore, mentioning drug screening advancements, mitochondrial functionality was restored using a compound identified from gene silencing of DGUOK whose use was employed in mtDNA depletion syndrome.[44]

It has made the most needed advancements in terms of SARS Cov-2 treatment with this ongoing pandemic with the core idea of imitating lungs with the same physiology and composition as in humans and their controlled introduction to viruses' especially novel Corona virus 2019. Afterwards genes which take part in entry and functioning of viruses, as well as the genes that have pivotal role in generating prudent and preferable immune response against the introduced virus are studied. The same can be regulated, for therapeutic interventions and effective treatment.

The model also acts as a guide to enlist etiological and possible risk factors that make the organ susceptible towards developing an infection and also helps in exploring various protective mechanisms that can efficiently combat the intruder present. The most recent execution of this technology was carried out in covid-19, which selectively targeted the RNA sequence and inhibited its multiplication ability. Therefore this technique can be the potential answer for resistant as well as yet immortal viruses hindering living forms.[44]

In terms of Eukaryotic organisms, fungi with numerous filaments are used for antibiotic and various acids (which are organic in nature) production. Some species are used as models for research and development purposes. They aid in the production of important and biologically useful enzymes employed in the production of food, textile and feed as well as a wide variety of fermented eatables and beverages. So this technique was employed to genetically modify the coding of these organisms to elevate production scale and achieve excellence in the same. So now, they are proficiently referred to as bio factories. This has increased production capacity of antibiotics while making it economical too. Other secondary metabolites critical for living organisms can be precisely obtained also.[48]
In terms of agricultural advancements, various comprehensive research has been taken place all around the globe to increase salt tolerance amongst different breeds of rice. This gene editing tool has been successfully used to achieve the above mentioned objective.[49]

So we can confidently testify that CRISPR Technology is indeed nobel in nature, with its wide array of applications and it has been proven and to be an improvised, more efficient and precise gene modification tool. It's power can be harnessed with more focused and distinctive research, to fully gain access of this virtuous and sublime tech.

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