

# Magnum Opus: Clustered Regularly Interspaced Short Palindromic Repeats Biology and Prokaryotic Gene Silencing

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## ABSTRACT

Gene Silencing was a technology that was established in eukaryotic system a decade ago and is being used as a research tool widely. However, prokaryotic gene silencing was not workable, till recently a team of researchers from the University of Georgia have proved it possible. Where they have shown that short motif sequences determines the targets of the prokaryotic Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) defence system is regulated by RNA guided Cas protein complex. Thus role of CRISPR system in microbial defense against foreign genetic material (Plasmid or Phages) is an important milestone in the field of microbial molecular biology/biotechnology. These findings will make it easier to understand the significance of a gene, metabolically or physiologically. The revelation by this novel finding by core group of researcher is indeed, Magnum opus. This article is a commentary, to bring to light, prokaryotic gene silencing as one of the latest advances in prokaryotic science.

**Keywords:** Gene Silencing, Prokaryotes, CRISPR, RNAi, Microbial Immunity

## 1. INTRODUCTION

For all of us, working to unfold the puzzling and intriguing world of genes in prokaryotes, their sequential makeup and their significance, have reasons to breathe easy and stretch, now. Gene Silencing a promising technology that fetched Nobel Prize to the discoverers Craig Mello and Andrew Fire, that was confined to eukaryotic system, ever since its discovery in 1998, is now feasible in prokaryotic systems (Hale *et al.*, 2012). Hale *et al.* (2012) supported by state that, rationally designed RNAs can work with bacterial immunity to silence target genes in prokaryotes (Rusk, 2012). It's time to crack the code!

Understanding the physiological or metabolic role of a gene was/is crucial to many of biological processes. Till the recent revelations on prokaryotic gene silencing, the role of a gene in a prokaryotic system could be determined either by 'knout-out' or

by 'mutational studies'. RNA interference (RNAi/Post-transcriptional Gene Silencing) could not be applied to prokaryotes.

### 1.1. Gene Silencing Basics

Post-transcriptional gene modification and its silencing is mediated by short interfering RNA (siRNA). This is believed to be a naturally occurring biological process, which is conserved in plants and animals (Keates *et al.*, 2008). The innate nature of RNAi owing to post-transcription rather than transcription level which has been adopted from dsRNA through the suppression of epigenetic or against intronic regions corresponding to transcribed (Fire *et al.*, 1998; Hammond *et al.*, 2001). RNA interference (RNAi) in mammalian cells otherwise termed as 'Gene Silencing' has been stated to have evolved as an immune response to viral invasion (Caplen *et al.*, 2001; Hammond *et al.*, 2001). This understanding led to the discovery of artificially synthesized siRNA and

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synthetic siRNA as we know, that revolutionized our understanding to the functions of genes.

Gene silencing that have described in prokaryotes is different from the gene silencing that happens in eukaryotic system, with reference to exogenous siRNA (Hale *et al.*, 2008; 2009; 2012). In the eukaryotes, when siRNA molecules are applied exogenously, the siRNA duplexes are unwound by RNA-Induced Silencing Complex (RISC). In this process, the sense strand, homologous to the target gene transcript (mRNA) is eliminated. The remaining anti-sense strand that is complementary to the target gene transcript, along with the RISC complex, binds to the transcript. The RISC complex binds to the complementary region of the target mRNA through the antisense siRNA strand. RISC complex then cleaves the target mRNA molecule using Argonaute-2 protein activity (Hale *et al.*, 2008; Wiedenheft *et al.*, 2011a; 2011b; 2012).

### 1.2. The ‘Crispr’ Purpose in Prokaryotes

Microbes have a unique defensive mechanism through CRISPR system, which acts as a determinant of genetic interference pathway against phages and plasmids (Labrie *et al.*, 2010; Garneau *et al.*, 2010; Sapranauskas *et al.*, 2011). Since viruses show diverse antigenic variations (Rohwer, 2003; Pignatelli *et al.*, 2008; Hambly and Suttle, 2005) caused due to rapid mutation, they easily evade the host immune system. Immunity to these viral DNA is a challenging issue and hence CRISPR-cas system participating in disintegration and immobilization of their gene activity, a subject of interest. Through specific nuclease activity of CRISPR associated Cas the foreign DNA/plasmid is spliced into smaller fragments. These fragments are then transcribed. The transcribed sequences are further utilized as non-coding RNA template for interference, invasion and disintegration (Hale *et al.*, 2009). Thus immunity in microbes is a RNA-encoded memory response, which is always acquired and adaptive (Barrangou *et al.*, 2007; Deveau *et al.*, 2008; Koonin and Makarova, 2009; Horvath and Barrangou, 2010; Marraffini and Sontheimer, 2010; Bhaya *et al.*, 2011; Shah and Garrett, 2011). Unlike, in eukaryotes, innate response is not at all necessary in prokaryotes to achieve protection from invasion of such foreign particles. Because of their unique CRISPR system, they do not require prior contact of these foreign particles in order to react against them. CRISPR-Cas system a new-generation biological tool for the development of gene-based targeted therapy is an immune mechanism adopted by

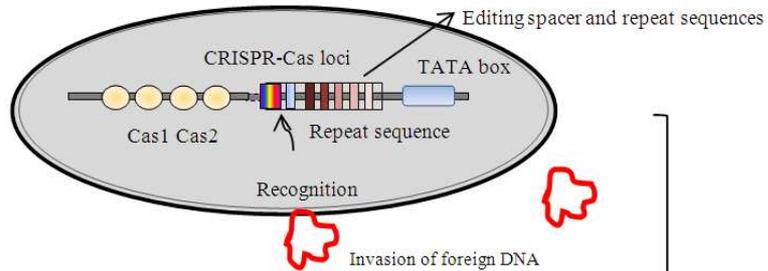
microbes against invading nucleic acid either in the form of plasmids or encapsulated in a virus.

### 1.3. The ‘Crispr-Cas’ Mechanism

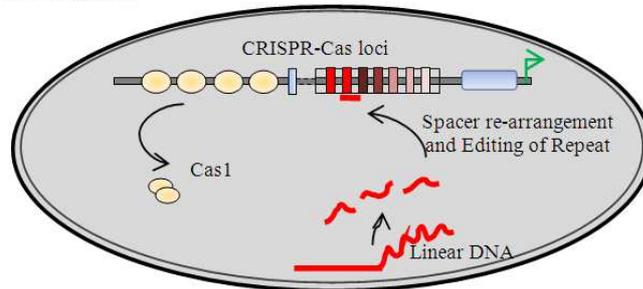
CRISPR-Cas-mediated defense mechanism has been widely discussed in terms of immunization by controlling invasion and integration of foreign particles and immunity (Horvath and Barrangou, 2010; Babu *et al.*, 2011) through the development of inherited memory (Oost *et al.*, 2009). However, in the larger interest it is worthwhile discussing the stage-wise mechanism of the system. Previous data reveals that CRISPR-Cas mediates both active and passive immunity through different immunization process (**Fig. 1**). The process involves (i) recognition of invasive particles, (ii) propagation of repeat sequence selection of spacer sequence and integration into CRISPR loci, (iii) transcription, includes editing of spacer sequence and synthesis of multiple copies of non-coding RNA sequence with the help of multifunctional nucleases, Cas1, Cas2, Cas3, finally, (iv) Cas specifically cleaves and splice these invasive foreign phage DNA or plasmid and acquire immunity against invasive genetic materials is thus developed (Wiedenheft *et al.*, 2009; 2011a). In terms of active immunity host directly comes in contact with these foreign particles and integrates while passive immunity develops through transfer of predisposed acquired information from generation after generation.

CRISPR are regions in a prokaryotic genome that contains multiple short direct repeats separated by spacer genes (Hale *et al.*, 2009; 2012). CRISPR confers immunity in prokaryotes against invading exogenous genetic materials such as plasmid or phage (Mojica *et al.*, 2009). When exogenous genetic material invades the prokaryotic cell, they are processed by a group of genes that are associated with the CRISPR region. These genes are termed as the cas genes. The Cas proteins derived from the cas genes, break down the invading genetic material into small fragments of 25 to 30 nucleotide base pairs. These fragments are then inserted into the CRISPR region. RNA molecules (along with the exogenous inserts) derived of constitutive transcription of the CRISPR are further processed by Cas proteins to yield small RNA fragments composed of the exogenous genetic material flanked with a repeat sequences. These RNA fragments are complementary to the invading genetic material. These RNA fragments forms an effector complex with Cas proteins (similar to the RISC complex in eukaryotes). This effector complex, due to the complementary, binds and silences the invading exogenous genetic material (Hale *et al.*, 2012; Caplen *et al.*, 2001; Wiedenheft *et al.*, 2011b; 2012).

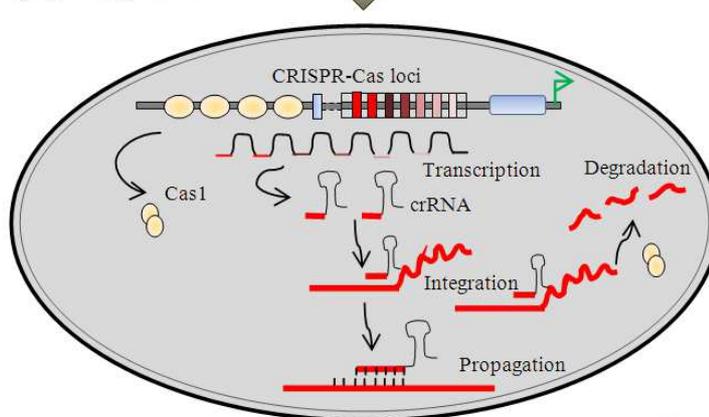
**Step 1: Recognition, Repeat Sequence Synthesis and Editing**



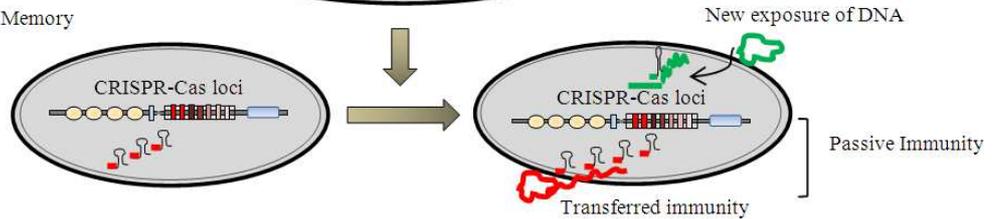
**Step 2: Spacer re-arrangement and Integration**



**Step 3: Transcription, Interloping and Degradation**



**Step 4: Immunity and Memory**



**Fig. 1.** CRISPR-Mediated active and passive immunity may achieve in four different steps: Step 1 includes recognition of invasive foreign DNA and synthesis of repeat sequences, step 2 includes re-arrangement of spacer and editing in repeat sequences with the help of multifunctional protein Cas1. Cas1 splices into small pieces are now transcribed into RNA encoded to these sequences and make copies of non-coded RNA that integrates with foreign DNA. Step3: These non-coding RNA utilizes to interfere in foreign DNA invasion and its degradation. Step 4: In this process microbes develops memory for specific DNA exposed to it in order to acquire immunity and transfer this immunity passively from one generation to another

#### 1.4. The Discovery

The idea about the Clusters of Regularly Palindromic Repeat (CRISPR) locus developed in (Mojica *et al.*, 1995) from the discovery of short sequence in between the 28-30 nucleotide (nt) long repeats in *Escherichia coli* K-12 chromosome (Ishino *et al.*, 1987; Nakata *et al.*, 1989). Since then, there have been several reports on cloning and sequencing of these genes in prokaryotes (Hermans *et al.*, 1991; Kawarabayasi, 1998; Kawarabayasi *et al.*, 1999; Hoe, 1999; Mojica *et al.*, 2000). However, its science and significance towards application have been established only after the year 2000 (Bolotin *et al.*, 2005; Mojica *et al.*, 2000; 2005; Pourcel *et al.*, 2005; Makarova *et al.*, 2006).

#### 1.5. Codicil

Ultimate goal of all medical research is to find out the target molecule as a key regulator of immune system by means of either activating host immune response or by inhibiting microbial response. This recent discovery about CRISPR system in bacteria and archaea brought revolution in the field of genetic engineering and biotechnology. Molecular mechanisms of these microbial effects are widely regulated by nucleic acid followed by alteration of hyper-variable gene patterns where non-coding RNA from CRISPR loci provides acquired immunity against non-specific sequence (Karginov and Hannon, 2010). Putative regulatory role of these non-coding RNA in CRISPR system provides important information about naturally controlled defensive mechanism, which may help in developing gene-specific model in medical research. Using this crisp CRISPR system, we can insert any target gene of interest that encoded for immunity by such regulated CRISPR may be achieved through siRNA or miRNA in eukaryotic animals or mammalian (Makarova *et al.*, 2006).

This technique of Gene silencing has proved to be a new-generation high throughput biological tool in understanding the transcriptional activity of janus-kinase (Jak)-Signal Transducer and Activator of Transcription (STAT) or Nuclear Factor-kappaB (NFκB) and also functional mechanism of other signalling pathway (Sharma and Rao, 2009).

## 2. CONCLUSION

Observation by Hale and his colleagues using Cmr-crRNA complexes, has given a workable solution to targeted destruction of gene transcripts (post-transcriptional) in prokaryotes.

This finding is an important milestone in the field of microbial molecular biology/biotechnology, as it will make it easier to understand the significance of a gene, metabolically or physiologically. The revelation by this novel finding by core researcher is indeed, Mangum opus. It is high time researchers in the domain, young and old alike, embraced this technology for deciphering the significance of genes and the information contained in them.

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