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Current Opinion in Immunology

The roles of CRISPR–Cas systems in adaptive immunity and beyond

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Clustered regularly interspaced short palindromic repeats (CRISPR) and accompanying Cas proteins constitute the adaptive CRISPR–Cas immune system in bacteria and archaea. This DNA-encoded, RNA-mediated defense system provides sequence-specific recognition, targeting and degradation of exogenous nucleic acid. Though the primary established role of CRISPR–Cas systems is in *bona fide* adaptive antiviral defense in bacteria, a growing body of evidence indicates that it also plays critical functional roles beyond immunity, such as endogenous transcriptional control. Furthermore, benefits inherent to maintaining genome homeostasis also come at the cost of reduced uptake of beneficial DNA, and preventing strategic adaptation to the environment. This opens new avenues for the investigation of CRISPR–Cas systems and their functional characterization beyond adaptive immunity.

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Current Opinion in Immunology 2015, **32**:36–41

This review comes from a themed issue on **Innate immunity**

Edited by **Zhijian J Chen** and **Sebastian Amigorena**

For a complete overview see the Issue and the Editorial

Available online 6th January 2015

http://dx.doi.org/10.1016/j.coi.2014.12.008

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Introduction

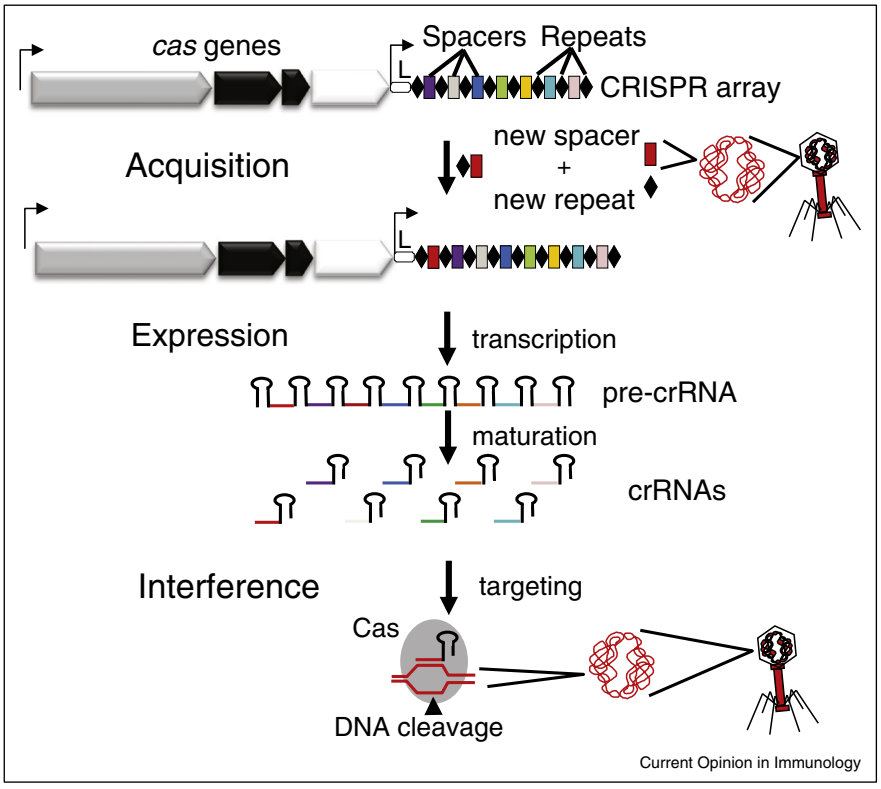
The ability to withstand viral predation is a hallmark of survival for most life forms. Over time, bacteria have managed to thrive in a diversity of inhospitable habitats, despite challenging environmental conditions, competition for scarce resources, and predation from other life forms such as viruses. Much of the sustainable success of bacteria lies in their concurrent ability to flexibly expand their genetic repertoire, dynamically manage genome homeostasis, and fend off viruses using a plethora of defense systems. Noteworthy, eubacteria have managed to control the size of their genomes by balancing tactical acquisition of beneficial material with strategic loss of extraneous and redundant genes, in a dynamically choreographed dance with invasive mobile genetic elements such as plasmids, viruses, and transposons [1]. Remarkably, bacteria have managed to orchestrate this without the genome expansion observed in the most advanced eukaryotes, in which a large proportion of the genome consists of extraneous and redundant repeated elements derived from exogenic elements. The recently discovered clustered regularly interspaced short palindromic repeats (CRISPR) and associated proteins (Cas) have been shown to provide immunity against viruses and plasmids in bacteria and archaea, in an adaptive manner, and has been established as a critical guardian of bacterial genomes [2–4]. However, several recent studies have shed light on additional functional roles of CRISPR–Cas systems, beyond immunity. These novel roles expand the functional repertoire of CRISPR–Cas systems and highlight the need to manage tradeoffs between safe-keeping genome integrity, and acquisition of beneficial mobile genetic elements for adaptive purposes.

CRISPR–Cas systems provide adaptive immunity in bacteria

Generally, CRISPR–Cas immune systems function in three distinct stages that provide DNA-encoded [5••], RNA-mediated [6••], sequence-specific targeting of exogenous nucleic acids [7••,8] (Figure 1). Specifically, as part of the *adaptation* process, pieces of DNA are sampled from invasive mobile genetic element and acquired as novel 'spacers' into CRISPR loci, to build immunity and immune memory [5••]. Subsequently, during the *expression* process, this repeat-spacer array is transcribed and processed into small interfering CRISPR RNAs (crRNAs) [6••]. In the *interference* stage, these guide RNAs direct Cas endonucleases for sequence-specific targeting, cleavage and degradation of complementary nucleic acid [9••,10]. In the past five years, numerous studies have unraveled the molecular basis of the CRISPR–Cas systems mechanism of action for adaptive immunization, biogenesis of small interfering RNAs, and sequence-specific targeting and cleavage of viral and plasmid DNA [2–4]. Although these systems are generally split into three different major types and 11 subtypes, the general molecular principles that drive spacer-based vaccination, crRNA biogenesis and target nucleic acid cleavage are shared across CRISPR–Cas systems, with most differences observed in the crRNA:Cas ribonucleoprotein complexes that drive targeting and cleavage [11•].

Several studies investigating the role of CRISPR–Cas systems in the arms race between bacteria and their

Figure 1



CRISPR–Cas immune systems. CRISPR-encoded immunization and interference. In the adaptation stage, exogenous DNA is sampled and a novel spacer is integrated into the CRISPR locus; in the expression stage, the CRISPR array is transcribed and processed into small interfering CRISPR RNAs (crRNAs) that guide Cas endonucleases towards target complementary DNA in the interference stage.
Adapted from [49].

viruses have established CRISPR as a *bona fide* adaptive immune system [12–15]. An intriguing recent study showed that CRISPR-mediated vaccination shares fundamental features with mammalian immunity, as infection by defective bacteriophage was shown to drive rapid CRISPR immunization [16]. Specifically, it was shown that attenuated viruses unable to replicate due to ultraviolet (UV) exposure or restriction–modification (R–M) can trigger CRISPR immunization. This is reminiscent of the use of inactivated and deficient viruses and bacteria for human vaccination.

Evolutionarily, these molecular machines provide adaptive immunity against invasive genetic elements in a Lamarckian manner, given the inheritable nature of the acquired spacers that provide beneficial vaccination. Intriguingly, CRISPR–Cas systems also encompass Darwinian features, with seemingly random spacer acquisition at the population level, followed by selection of surviving clones with competitive fitness [17]. Some of the CRISPR–Cas features are also reminiscent of mammalian adaptive immunity, with essential features encompassing specificity (ability to carry out sequence-based targeting), diversity (capacity to mount a response against diverse molecular invaders, with a multitude of sequences that can be sampled from exogenous elements) and memory (sequences acquired into CRISPR loci are passed on vertically to bacterial offsprings). These features have set the stage for natural genesis or engineering of variants with enhanced viral resistance in bacterial cultures of industrial interest. Noteworthy, CRISPR–Cas immunity can be naturally harnessed by phage challenge followed by targeted screening of isofunctional and isogenic CRISPR-immunized phage-insensitive mutants, an approach which has been broadly and successfully implemented for the vaccination of valuable commercial dairy starter cultures [18].

Repurposing the Cas machinery for genome editing and transcriptional control

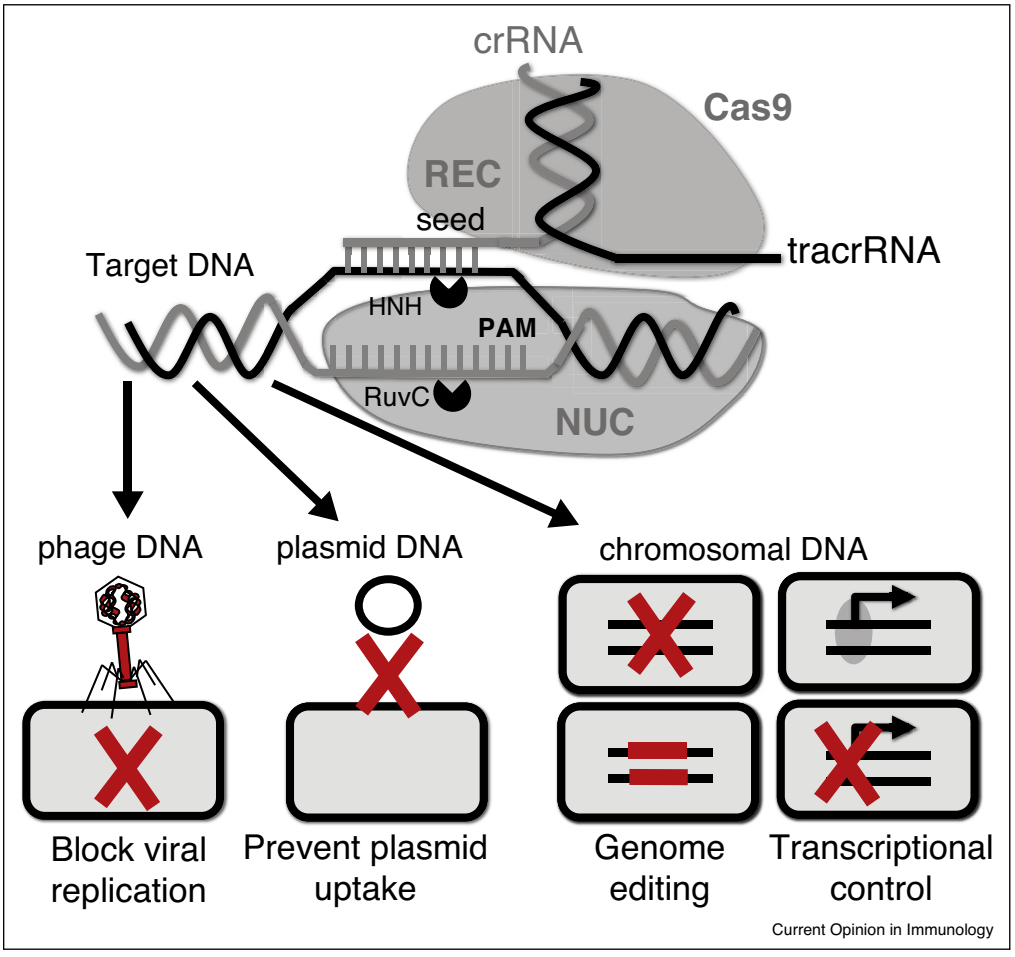
Notwithstanding the natural immune role of CRISPR–Cas systems in prokaryotes, the most visible and significant recent research has arguably been centered around the repurposing of the Cas machinery for genome editing and transcriptional control in eukaryotes [19,20]. Indeed, repurposing of the programmable Cas9 endonuclease has

revolutionized genome editing [21,22,23•] and open new avenues for transcriptional control with unprecedented ease and flexibility [24–29], as illustrated by the CRISPR craze witnessed since 2013 [30•].

In terms of genome editing, the Cas9 endonuclease can be programmed using native crRNA:tracrRNA duplexes or a homologous single guide sgRNA chimera [31•] that drive sequence-specific targeting of complementary DNA and genesis of a double stranded DNA break (DSB) by nicking of both DNA strands using HNH and RuvC domains [10,31•] (Figure 2). Double stranded breaks can then be repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR) to generate sequence mutations precisely at the DNA break point [21,22,23•]. Edits generated by error-prone NHEJ typically consist of single nucleotide polymorphisms (SNPs) and small insertion or deletions (INDELs) that are un-predictable in nature. In contrast, HDR-based edits can be precisely designed and engineered into a DNA recombination template typically provided in *trans*. A key advantage of HDR over NHEJ editing is the ability to engineer sequence insertions/additions and deletions/removals at the gene scale. The targeting precision, together with DSB-generating efficiency of Cas9, have rendered genome editing widely accessible, and re-invigorated gene therapy with stunning and un-anticipated speed [20,21].

With regard to transcriptional control, a de-activated form of Cas9 (dCas9), in which mutated nickase domains abrogate the endonuclease activity can be used

Figure 2



Cas-mediated DNA targeting and cleavage. The Cas9 endonuclease forms a ribonucleoprotein complex in combination with the dual guide RNA (crRNA and tracrRNA), and the target dsDNA. First, the Cas9:guide RNA complex binds to proto-spacer adjacent motif (PAM) and drives the formation of an R-loop in the target DNA for genesis of a double stranded break using the RuvC and HNH nickase domains [50]. The former primarily involves the recognition (REC) Cas9 lobe (top), and the latter is primarily driven by the nuclease (NUC) lobe (bottom). Cas-mediated targeting can aim at phage DNA for antiviral resistance (cleaved viral DNA cannot replicate), plasmid DNA to preclude the uptake and dissemination of plasmids (cleaved plasmid DNA cannot replicate), and chromosomal DNA for genome editing (insertion of mutations using endogenous DNA repair systems at the site of cleavage) or transcriptional control (dCas9 binding blocks RNA polymerase).

for transcriptional repression either by occluding RNA polymerase binding to promoter sequences, or fused with transcriptional repressors to block transcription [24–29]. Conversely, dCas9 can be fused to transcriptional activators to recruit RNA polymerase and activate gene transcription [24–29]. Although the *Streptococcus thermophilus* CRISPR–Cas systems and *Streptococcus pyogenes* Cas9 remain the best characterized and most utilized CRISPR models, respectively, novel systems are ever-increasingly studied. It is anticipated that the Cas9 arsenal will expand significantly in short order and will further expand the CRISPR toolbox for editing genomes and modulating their transcriptome.

The evolutionary cost(s) of playing defense

Original studies investigating the immune role of CRISPR–Cas systems in bacteria and archaea have provided insights into the co-evolutionary interplay between prokaryotes and their viruses in general, and their genetic arms race in particular [12–15,32–34]. Indeed, studies have shown that CRISPR loci rapidly diversify in populations exposed to viruses [14,17]. In response, bacteriophages rapidly evolve to specifically escape CRISPR-encoded immunity by mutating or even deleting highly targeted genome areas [15,33]. Ironically, a recent report has shown that in a twisted turn of events, phage can actually uptake and use bacterial CRISPR–Cas systems to target antiviral defense systems in their host, illustrating the evolutionary value of these systems [35]. This has provided insights into the high mutation rates of viral genomes, and the ecology of host–virus populations, with indications that CRISPR–Cas immune systems are a major driving force for bacterial sustainability and virus genome evolution.

Notwithstanding the ability to maintain genomic homeostasis by fending off invasive genetic elements, protecting the chromosome against exogenous elements actually can be a barrier against the uptake of genetic material that may be beneficial to the cell [36–40]. Indeed, phage, plasmids and conjugative elements have been known to occasionally carry beneficial genes that positively contribute to bacterial adaptation and provide evolutionary benefits that increase fitness, such as antibiotic resistance and virulence factors. As recently reported, CRISPR targeting of bacterial chromosome sequences can lead to loss of pathogenicity islands [41]. This is consistent with the spread of plasmid encoded-antibiotic resistance cassettes, and the horizontal gene transfer of pathogenicity islands between bacterial genomes. There are several instances in which a negative correlation has been established between the occurrence and diversity of CRISPR–Cas systems, and the presence of plasmids and prophages, as illustrated in *Campylobacter*, *Enterococcus* and many group A *Streptococcus* species [42]. In fact, CRISPR loci rarely occur in the genomes of *Enterococcus faecalis* and *Enterococcus faecium*, in which mobile genetic elements typically constitute up to 25% of the genome [39,42]. This can be related to the ability of CRISPR–Cas systems to directly interfere with natural transformation, as shown in *Straphylococcus* [7,37]. Specifically, CRISPR-based targeting of conjugative plasmids has been shown to detrimentally affect antibiotic resistance in *Staphylococcus epidermidis* [36,37]. Conversely, the paucity of CRISPR loci in *Staphylococcus aureus* likely correlates with the occurrence of plasmids, prophage and mobile genetic elements that increase virulence in this pathogen [36,42]. Similar findings have been reported in pneumococci, notably with experiments showing that CRISPR can prevent capsule switching for successful infection by *Streptococcus pneumoniae* [41]. As such, the cost associated with CRISPR-based genetic defense can lead to loss of key phenotypes, such as virulence in human pathogens. This trade off is a likely explanation for the occurrence of CRISPR–Cas systems in 'only' half of bacteria. Furthermore, the occurrence and activity of CRISPR–Cas immune systems likely reflect the frequency and richness of predators and invaders in the ecosystem, the intensity of the CRISPR-mediated virus–host arms race, as well as the presence of alternative defense systems in the host, such as R–M, toxin–antitoxin, surface receptor mutation and abortive infection.

CRISPR roles beyond adaptive immunity

Although antiviral vaccination has been shown for various CRISPR–Cas systems, several recent studies have unraveled functions beyond adaptive immunity [43]. Actually, a growing body of work is shedding light on the costs and benefits of CRISPR–Cas systems, which has expanded our understanding of the various roles they play in bacteria. A new perspective has emerged on CRISPR functions, based on the ability to control transcription endogenously, and regulate important lifestyle-based bacterial phenotypes such as pathogenicity. Indeed, a recent study showed that CRISPR–Cas systems can regulate endogenous genes that drive virulence and pathogenesis of *Francisella novicida* [44••,45]. Specifically, a Type II CRISPR–Cas systems was shown to repress transcription of a bacterial lipoprotein which typically triggers a pro-inflammatory response in the human host. Ironically, this bacterial immune systems tinkers with regulation of genes that encode factors that direct bacterial pathogenesis, and allows the bacterium to use the CRISPR immune system to escape the human immune system. Likewise, it was recently shown in *Campylobacter jejuni* that CRISPR can alter pathogenic behaviors [46]. Similar findings have implicated CRISPR–Cas systems in *Neisseria meningitidis* pathogenesis [47] and *Legionella pneumophila* infection [48].

Overall, these emerging studies establish additional functional roles for CRISPR–Cas systems that are important for virulence of bacteria, and regulation of pathogenic phenotypes.

Perspective

Altogether, the relationship between CRISPR and their hosts can be 'complicated' at times, with the need to strategically balance tradeoffs between limiting susceptibility to invasive mobile genetic elements on one hand (maintaining genome integrity), and the ability to uptake valuable exogenous DNA on the other hand (allowing genome flexibility). As further studies investigate the function(s) of CRISPR–Cas systems in a variety of organisms and model systems, the extent of their role beyond adaptive immunity will be rightfully determined. Given the ever-increasing interest in CRISPR–Cas immune systems in general, and Cas9-based applications in particular, the characterization of the molecular underpinning of these fantastic molecular machines will set the stage for further exploitation of the Cas machinery for immunity, genome editing, transcriptional control and beyond.

Conflicts of interest

RB is a co-inventor on several patents related to CRISPR–Cas systems and their various uses. RB is also on the board of directors of Caribou Biosciences, and a co-founder of Intellia Therapeutics.

Acknowledgements

RB is supported by start up funds from North Carolina State University. The author would like to thank his many colleagues and collaborators in the CRISPR field for their insights into these fantastic molecular systems.

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