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Advancing the design and delivery of CRISPR antimicrobials

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Abstract

CRISPR-Cas systems are prokaryotic immune systems whose RNA-guided nucleases have been co-opted for applications ranging from genome editing and gene regulation to *in vitro* diagnostics and DNA imaging. Here, we review the current efforts toward repurposing CRISPR nucleases as programmable antimicrobials. Antimicrobial activity is achieved by targeted cleavage of multidrug-resistance plasmids or the bacterial chromosome, resulting in antibiotic sensitivity or cell death. As part of the review, we discuss the different types of nucleases available for CRISPR antimicrobials, the use of bacteriophages as delivery vehicles, and opportunities to enhance antimicrobial activity, delivery, and specificity. Through further advances, these programmable DNA-targeting antimicrobials may help quell the spread of antimicrobial resistance and provide a tool for the manipulation of complex microbial communities.

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Introduction

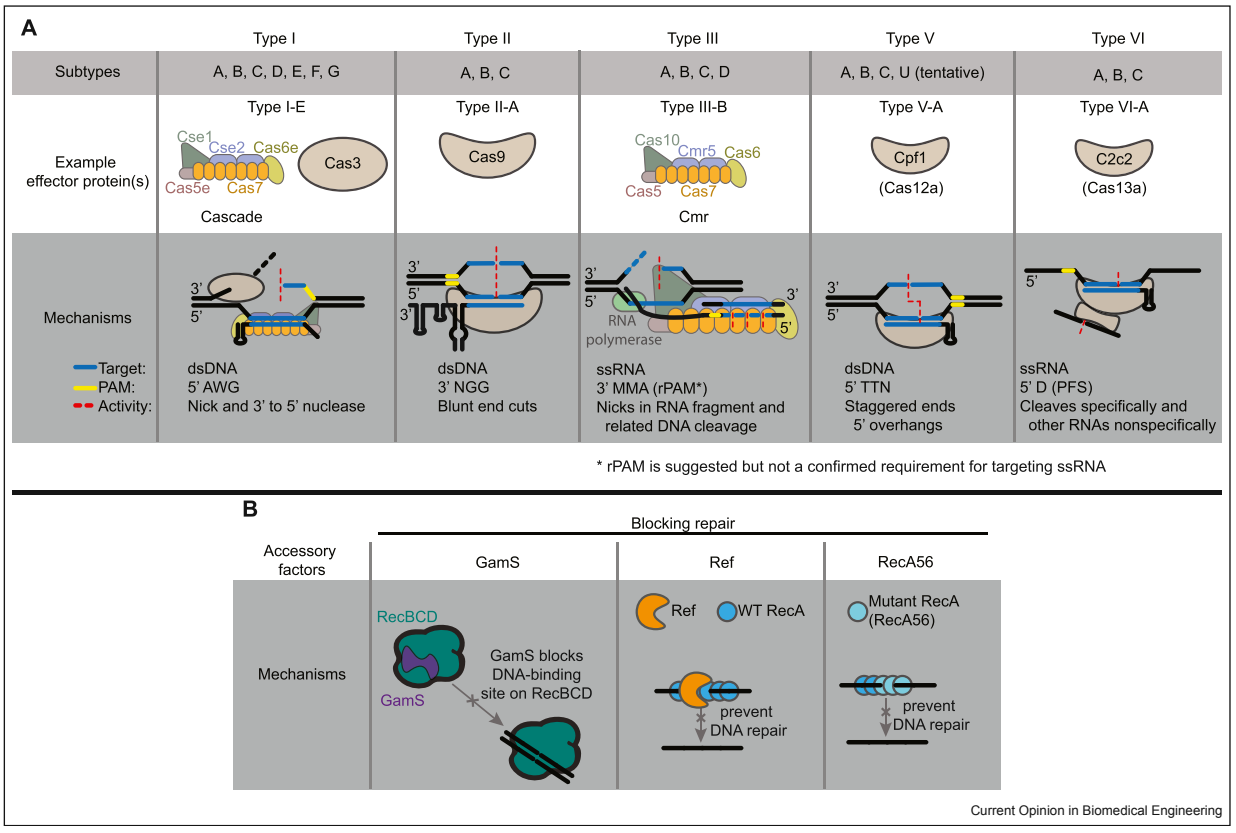
CRISPR-Cas systems are adaptive immune systems in bacteria and archaea whose CRISPR nucleases and guide RNAs have formed the foundation of powerful tools in biotechnology and medicine. The guide RNAs direct the nuclease to bind and cleave complementary DNA or RNA sequences often flanked by a protospacer-adjacent motif (PAM). The ease of designing guide RNAs, the portability of the nuclease-guide RNA pair, and the broad applicability of programmable DNA binding and cleavage has given rise to a diverse set of applications ranging from genome editing and gene regulation to *in vitro* diagnostics, real-time DNA and RNA imaging, and gene drives [1,2].

The standard mechanism of CRISPR-based genome editing is the directed repair of cleaved DNA. In eukaryotic cells, the cleaved DNA is efficiently repaired through ubiquitous mechanisms such as homology-directed repair or non-homologous end joining. However, in bacteria, multiple studies have indicated that DNA cleavage by CRISPR nucleases often cannot be repaired and is therefore lethal. One line of evidence is the dearth of naturally occurring genome-targeting spacers in active CRISPR-Cas systems, even though incorporation of such sequences regularly occurs during naive spacer acquisition [3,4]. Separately, the introduction of genome-targeting guide RNAs in bacteria with an active CRISPR-Cas system arrested the cell cycle, leading to eventual death or loss of large genomic segments containing the target sequence [5,6]. These insights helped inspire the use of CRISPR nucleases as programmable antibacterial agents that could programmably and irreversibly destroy targeted DNA only in selected bacteria. This mechanism has been developed primarily to address the rising challenge of antibiotic resistance and the diminishing supply of new small-molecule drugs [7]. Here, we review how CRISPR nucleases have been harnessed as programmable antimicrobial agents to combat human disease and the rise of antibiotic resistance, and we discuss opportunities for the further development of this promising antimicrobial strategy.

CRISPR nuclease selection

CRISPR-Cas systems commonly act as immune systems by cleaving foreign DNA or RNA, yet a diversity of system types have been reported (Figure 1A) [8–10]. These system types are differentiated based on the associated proteins responsible for acquisition and targeting as well as their modes of action. For instance, Type II systems generate blunt-ended cuts in target DNA, Type III systems synergistically cleave target RNA and DNA, and Type VI systems cleave target RNA followed by the non-discriminate cleavage of other RNAs [10–12]. Given that CRISPR-Cas systems are present in roughly half of bacteria, these endogenous systems could be co-opted as antimicrobials through the delivery of a guide RNA targeting the bacterial genome

Figure 1



CRISPR antimicrobial design. (**A**) Diversity of CRISPR-Cas systems with their respective nucleic-acid targets and mechanisms of attack. Each displayed PAM is from a representative, well-characterized system. Type IV systems have not been experimentally characterized and are therefore excluded here. (**B**) Potential accessory factors for augmenting CRISPR antimicrobials by blocking DNA break repair.

[5,13]. However, the utility of this approach is limited to bacteria with functionally active CRISPR-Cas systems and is sensitive to the expression and genetic stability of the CRISPR-Cas locus. A more robust and self-contained antimicrobial design consists of introducing the designed guide RNA and the CRISPR nuclease, the strategy used for virtually all CRISPR antimicrobials to-date.

While each type of nuclease has potential as an antimicrobial agent, the primary nuclease of choice has been Cas9, the effector protein from Type II CRISPR-Cas systems. Cas9 is the most thoroughly investigated RNA-guided nuclease and has been the trailblazer for current CRISPR technologies [14–18]. Cas9 has also been the primary nuclease used in CRISPR antimicrobials, where it has been successfully used to target antibiotic-resistance genes encoded on plasmids or in the genome [19,20] as well as essential and non-essential chromosomal genes [5,13,21,22].

One critical feature of Cas9 is that it generates a blunt-end double-stranded break that is amenable to DNA repair, potentially compromising Cas9 as an antimicrobial. Cui and Bikard directly reported this phenomenon in *Escherichia coli*, where cleavage events were often repaired by homologous recombination with extra copies of the genome [21]. The repair was dependent on RecA and allowed the cells to survive attack when Cas9 targeted a broad range of non-essential and even a few essential genes. What remains unclear is why some sites led to potent killing and whether repair will be a barrier to Cas9-based antimicrobials in other bacteria. Non-specific cytotoxicity from Cas9 has also been reported [23,24], raising concerns about potential non-specific killing in any cells that express exogenous Cas9.

Despite the intense focus on Cas9, Type I CRISPR-Cas systems have also shown promise as antimicrobial agents. The Cas3 nuclease for these systems nick and processively degrade the non-bound strand of DNA in the 3′-to-5′ direction, leading to immediate destruction of large portions of DNA upstream of the target sequence [25,26]. Accordingly, multiple groups have co-opted the I-E CRISPR-Cas system from *E. coli* for potent and selective killing. Gomaa et al. demonstrated the utility of the *E. coli* I-E system as a programmable