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Review

Harnessing the Prokaryotic Adaptive Immune System as a Eukaryotic Antiviral Defense

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Clustered, regularly interspaced, short palindromic repeats – CRISPR-associated (CRISPR-Cas) systems – are sequence-specific RNA-directed endonuclease complexes that bind and cleave nucleic acids. These systems evolved within prokaryotes as adaptive immune defenses to target and degrade nucleic acids derived from bacteriophages and other foreign genetic elements. The antiviral function of these systems has now been exploited to combat eukaryotic viruses throughout the viral life cycle. Here we discuss current advances in CRISPR-Cas9 technology as a eukaryotic antiviral defense.

The Prokaryotic Immune System

Prokaryotic cells possess innate and adaptive immune systems that conceptually parallel those found in eukaryotic organisms [1]. Prokaryotic restriction endonucleases function as an 'innate' immune defense mechanism, recognizing conserved nucleotide sequences and subsequently cleaving foreign nucleic acids. These endonucleases have formed the platform for the generation of powerful tools such as recombinant DNA technology and genome sequencing. Alternatively, CRISPR-Cas systems serve as the 'adaptive' immune system of bacteria and archaea. By incorporating short sequences of bacteriophage-derived or other foreign nucleic acids into their own genome, prokaryotes use CRISPR-Cas systems to recognize new targets and degrade these invaders upon secondary encounter, analogous to a memory response in eukaryotic organisms [2].

Cas9-mediated Targeting of Bacteriophages

The clustered, repetitive sequences (CRISPR array) that form the central feature of CRISPR-Cas systems were first discovered in 1987 in *Escherichia coli* [3], although it was not until 2007 that the function of CRISPR sequences and their conserved, adjacent *cas* genes (CRISPR-associated genes) was first described. Barrangou and colleagues demonstrated that upon bacteriophage infection, *Streptococcus thermophilus* integrated phage genomic sequences into the CRISPR array, and that these sequences, in conjunction with the *cas* genes, provided protection from subsequent viral challenge [4]. CRISPR-Cas systems have now been identified in over 90% of sequenced archaea, as well as roughly 50% of bacterial species [5,6]. The class II CRISPR-Cas9 system has been the most extensively studied and is discussed in detail below.

There are two distinct stages of Cas9-mediated immunity (Figure 1). During the acquisition phase, a bacterium encounters foreign nucleic acid, such as that of a phage genome. A portion of the phage genome is incorporated into the CRISPR array, and is termed a spacer [4,7,8]. The subsequent immunity phase occurs when the bacterium again encounters an identical foreign nucleic acid and proceeds to catalyze its cleavage. In this phase, Cas9 is guided by two small RNAs, the trans-activating RNA (tracrRNA) and the CRISPR RNA (crRNA) [9,10]. When the associated crRNA, transcribed from the CRISPR array, has complementarity to the invading

Trends

Cas9 technology has been utilized to inhibit pathogenic DNA and RNA viruses in cell culture. This has been accomplished by modulating expression of host factors required for viral entry, directly targeting virus genomes, transcriptionally activating antiviral genes, and cleaving the DNA stage of viruses that integrate into the host cell chromosome.

The Cas9 machinery has been shown to be efficacious in restricting pathogenic viruses in small rodent models.

Transgenic plants have recently been developed that inhibit the replication stage of geminiviruses, a major cause of crop losses worldwide.

Cas9 technology is a promising tool for the generation of virus-resistant transgenic plants and animals, as well as antiviral therapeutics.

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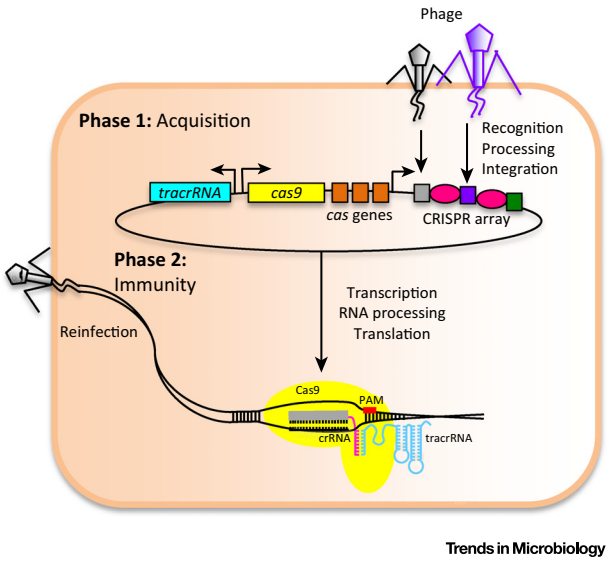

Figure 1. Cas9-Mediated Immunity in Type II CRISPR-Cas Systems. Phase 1: Acquisition. Upon encounter with foreign nucleic acid, such as that of a bacteriophage genome, a short sequence of the phage DNA is recognized, processed into small portions, and integrated into the CRISPR array by Cas proteins. Each sequence, termed a spacer, (gray, purple, and green boxes) is incorporated between identical CRISPR repeats (pink ovals). Phase 2: Immunity. In this stage, the bacterium encounters an identical foreign nucleic acid. Two small RNAs, the crRNA and the tracrRNA, guide the Cas9 effector protein to the phage genome. If there is sufficient sequence complementarity between the crRNA and the phage genome and a protospacer adjacent motif (PAM) is present, Cas9 then cleaves both strands of the target.

phage sequence, the two Cas9 endonuclease domains (RuvC and HNH) mediate cleavage of the targeted sequence. Endonuclease activity also requires that a short sequence on the foreign DNA adjacent to that bound by the crRNA, known as the protospacer adjacent motif (PAM), is recognized by Cas9 [10] (Figure 1).

In addition to its natural role within bacteria, the Cas9 system has been harnessed for diverse applications in eukaryotic cells. Cas9 can be programmed to target and cleave double-stranded DNA (dsDNA) sequences of interest by engineering single chimeric guide RNAs (gRNAs) composed of portions of the tracrRNA and crRNA [10] (Figure 2). Target specificity is achieved by simply modifying the short spacer region of the gRNA to a sequence complementary to the target. Coexpression of the gRNA with Cas9 in the cell of interest leads to target cleavage. Genomic alterations have already been performed in diverse cell types, including those from zebrafish [11,12], mice [13–15], humans [10,14,16,17], and an abundance of other organisms. Further, Cas9-based technologies have now been successfully exploited against eukaryotic viruses at different stages of their life cycle.

Cas9-mediated Inhibition of Eukaryotic Viruses

While directly targeting viral nucleic acids is an obvious strategy to inhibit viral replication, Cas9 has also been targeted to disrupt host factors critical for the viral life cycle. Additional strategies include using Cas9-transcriptional fusion proteins to reactivate viruses and render them susceptible to killing by the immune system, or to induce transcription of antiviral genes. Together, these avenues may provide effective means to inhibit, or even clear, viral infections. In this review, we discuss examples of pathogenic eukaryotic viruses that have been targeted by Cas9, as well as current obstacles, future implications, and questions regarding this technology.

HIV

More than 35 million people worldwide are infected with HIV [18]. HIV infects cells using the primary receptor CD4 [19] and chemokine coreceptors CCR5 [20] or CXCR4 [21]. If left untreated, infection can lead to progressive impairment and depletion of immune cells, particularly $CD4^+$ T cells, and AIDS [18]. Despite significant advances in antiretroviral regimens, treatment toxicity and viral resistance are limitations of current therapies. A vaccine remains elusive despite intense research effort [22–24].