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The multiplexed CRISPR targeting platforms

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The discovery and engineering of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) in the past several years have revolutionized biomedical research. The CRISPR technology showed great potential to advance detection, prevention, and treatment of human diseases in the near future. Compared to previous developed genome editing approaches, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), the CRISPR-based systems have numerous advantages. One example is that the CRISPR systems can be easily adopted to efficiently target multiple genes simultaneously. Several strategies and toolboxes have been developed to achieve multiplexed targeting using the CRISPR systems. In this short review, we will discuss the principle, approach, and application of these strategies.

Introduction

The CRISPR-associated systems were discovered in prokaryotes and shown later as adaptive immune systems to defend against viruses and plasmids [1,2]. In the infected cells, short DNA sequences originated from invading pathogens are stored in the form of "spacers" in the CRISPR array in the host genome [3]. CRISPR array is transcribed and processed into small RNAs containing a single spacer known as CRISPR RNAs (crRNAs) [4]. These crRNAs bind to CRISPR-associated (Cas) proteins and another small RNA molecule called trans-activating CRISPR RNA (tracrRNA), to form the effector complexes, which recognize foreign DNA or RNA sequences by base-pairing with spacer sequences in crRNAs [4,5] (Fig. 1a). Cas proteins, such as Cas9, then cleave the invaded target nucleic acid to achieve adaptive immunity response [2]. Since this discovery, extensive CRISPR-based tools for genome editing have been developed because the CRISPR systems provide a simple, quick, and efficient solution to manipulate gene expression. In fact, CRISPR engineering was one of the fastest developing fields in biology and medicine in the past five years. As one of the most successful stories, the powerful CRISPR tools have been applied to almost all model species, from bacteria to mammals, and have significantly accelerated the biological and biomedical research.

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The engineered CRISPR platforms

Streptococcus pyogenes Cas9 (SpCas9) was the first Cas system engineered to genome editing tools [6–8]. They include a Cas9 endonuclease and a single guide RNA (sgRNA), an artificial chimeric RNA molecule to comprise the function of crRNA and tracrRNA. sgRNA carries a direct sequence of ~20 nucleotides (nt) that pairs with DNA target, and Cas9 is in charge of generating a double-strand break (DSB) in a

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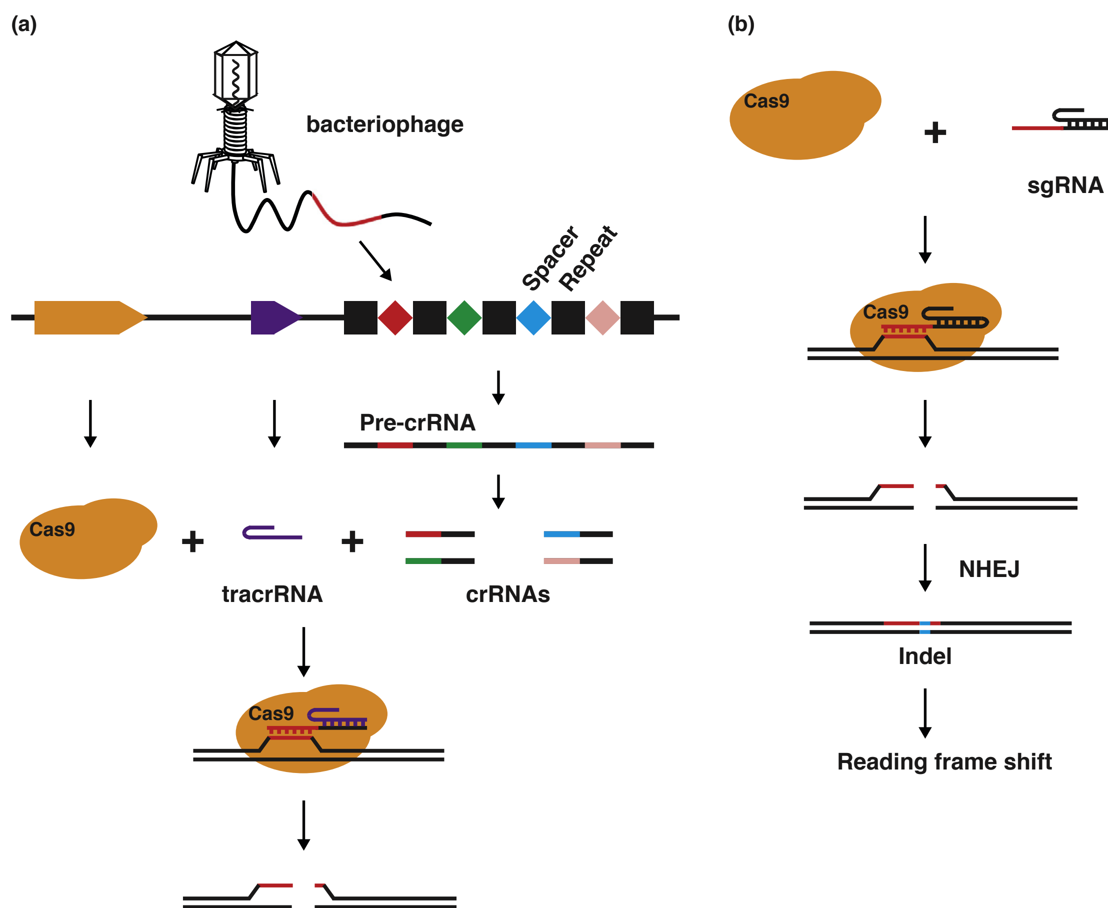

Fig 1. Natural and engineered CRISPR systems.
(a) Schematic of a natural CRISPR/Cas9 pathway. Foreign DNA is captured and inserted between repeats in a CRISPR locus in a bacteria genome. CRISPR array is transcribed and then processed into multiple crRNAs, each carrying a single spacer sequence and a repeat sequence. The crRNA forms a complex with Cas9 and tracrRNA and directs the complex to the foreign DNA carrying the same sequence as the spacer. Cas9 then generates a double strand break on the foreign DNA. (b) Schematic of an engineered Cas9 genome editing tool. It comprises a Cas9 endonuclease and a single guide RNA (sgRNA). Cas9 and sgRNA form a complex. The guide sequence on sgRNA directs the complex to the target site. Cas9 generates a double strand break, which is repaired via the error-prone non-homologous end-joining (NHEJ), leaving a small insertion or deletion (Indel) at the target site. Indels in protein coding region cause reading frame shift and early termination of protein translation, resulting in loss of protein expression.

targeted DNA locus [6] (Fig. 1b). Through the error-prone repair, DSB introduced by CRISPR/Cas9 abolishes the expression of selected proteins by shifting reading frames [6]. Because the SpCas9-based gene knockout tools are easy to use and highly efficient, they were quickly developed for applications in a wide range of species, including bacteria [9], yeast [10], plants [11,12], worms [13], flies [14], frogs [15], and mammals [16]. Since then, several distinct CRISPR-based tools have been developed based on natural CRISPR effectors. These enzymes include Cas9 from other species, such as FnCas9 [17], SaCas9 [18], St1Cas9 [19], St3Cas9 [20], NmCas9 [21], and other Cas proteins, such as Cas12a (Cpf1) [22] and Cas13a (C2c2) [23]. Additional Cas effectors are still under development for biotechnological applications [24].

Moreover, Cas proteins have been further developed to sequence-specific targeting platforms for a wide range of purposes in addition to generating DSB (Fig. 2). These purposes are achieved by fusing nuclease dead mutants of Cas (dCas) proteins with different functional domains [25]. For example, fusing dCas to transcriptional activation domains, such as VP64, or transcriptional repressors, such as KRAB, were used to alter the expression of genes [26–29]. Fusing dCas proteins to modulators of epigenetic markers can manipulate DNA methylation or histone modifications at the selected loci [25]. Additionally, fluorescent proteins fused to dCas9 provided a tool for visualizing native genomic loci in living cells [30].

Most of these studies have been performed using SpCas9, but it can be replaced with other Cas proteins. Furthermore,

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