Writing the manuscript introduction
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General background.

Almost all archaea and many bacteria achieve adaptive immunity through a diverse set of CRISPR-Cas (Clustered Regularly-Interspaced Short Palindromic Repeats and CRISPR-ASsociated proteins) systems, each of which consists of a combination of Cas effector proteins and CRISPR RNAs (crRNAs) (Makarova et al., 2011; Makarova et al., 2015).

Specific background (with all acronyms defined).

The defense activity of the CRISPR-Cas systems includes three stages: (i) adaptation, when a complex of Cas proteins excises a segment of the target DNA (known as a protospacer) and inserts it into the CRISPR array (where this sequence becomes a spacer); (ii) expression and processing of the precursor CRISPR (pre-cr) RNA resulting in the formation of mature crRNAs; and (iii) interference, when the effector module – either another Cas protein complex or a single large protein – is guided by a crRNA to recognize and cleave target DNA (or in some cases, RNA) (Horvath and Barrangou, 2010; Sorek et al., 2013; Barrangou and Marraffini, 2014). The adaptation stage is mediated by the complex of the Cas1 and Cas2 proteins, which are shared by all known CRISPR-Cas systems, and sometimes involves additional Cas proteins. Diversity is observed at the level of processing of the pre-crRNA to mature crRNA guides, proceeding via either a Cas6-related ribonuclease or a housekeeping RNaseIII that specifically cleaves double stranded RNA hybrids of pre-crRNA and tracrRNA. Moreover, the effector modules differ substantially among the CRISPR-Cas systems (Makarova et al., 2011; Makarova et al., 2015; Charpentier et al., 2015). In the latest classification, the diverse CRISPR-Cas systems are divided into two classes according to the configuration of their effector modules: Class 1 CRISPR systems utilize several Cas proteins and the crRNA to form an effector complex, whereas Class 2 CRISPR systems employ a large single-component Cas protein in conjunction with crRNAs to mediate interference (Makarova et al., 2015). Multiple Class 1 CRISPR-Cas systems, which include the type I and type III systems, have been identified and functionally characterized in detail, revealing the complex architecture and dynamics of the effector complexes (Brouns et al., 2008; Marraffini and Sontheimer, 2008; Hale et al., 2009; Sinkunas et al., 2013; Jackson et al., 2014; Mulepati et al., 2014). Several Class 2 CRISPR-Cas systems have also been identified and experimentally characterized, but they are all type II and employ homologous RNA-guided endonucleases of the Cas9 family as effectors (Barrangou et al., 2007; Garneau et al., 2010; Deltcheva et al., 2011; Sapranauskas et al., 2011; Jinek et al., 2012; Gasiunas et al., 2012).

Knowledge gap.

A second, putative Class 2 CRISPR system, tentatively assigned to type V, has been recently identified in several bacterial genomes (Schunder et al., 2013; Vestergaard et al., 2014; Makarova et al., 2015). The putative type V CRISPR-Cas systems contain a large, ~1,300 amino acid protein called Cpf1 (CRISPR from Prevotella and Francisella 1). It remains unknown, however, if Cpf1-containing CRISPR loci indeed represent functional CRISPR systems.

Why reader should care.

Given the broad applications of Cas9 as a genome engineering tool (Hsu et al., 2014; Jiang and Marraffini, 2015), we sought to explore the function of Cpf1-based putative CRISPR systems.

Statement of findings.

We found that the PAM for FnCpf1 is located upstream of the 5′ end of the displaced strand of the protospacer and has the sequence 5′-TTN. […]

Here we show, with preview of results.

Here we show that Cpf1-containing CRISPR-Cas loci of Francisella tularensis subsp. novicida U112encode functional defense systems capable of mediating plasmid interference in bacterial cells guided by the CRISPR spacers. Unlike Cas9 systems, Cpf1-containing CRISPR systems have three features: First, Cpf1-associated CRISPR arrays are processed into mature crRNAs without the requirement of an additional trans-activating crRNA (tracrRNA) (Deltcheva et al., 2011; Chylinski et al., 2013). Second, Cpf1-crRNA complexes efficiently cleave target DNA proceeded by a short T-rich protospacer adjacent motif (PAM), in contrast to the G-rich PAM following the target DNA for Cas9 systems. Third, Cpf1 introduces a staggered DNA double stranded break with a 4 or 5-nt 5′ overhang.
To explore the suitability of Cpf1 for genome editing applications, we characterized the RNA-guided DNA targeting requirements for 16 Cpf1-family proteins from diverse bacteria, we identify two Cpf1 enzymes, from Acidaminococcus sp. BV3L6 and Lachnospiraceae bacterium ND2006, that are capable of mediating robust genome editing in human cells.

Why reader should care.

Collectively, these results establish a Class 2 CRISPR-Cas system that includes an effective single RNA-guided endonuclease with distinct properties that has the potential to substantially advance our ability to manipulate eukaryotic genomes.

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