**Writing the manuscript introduction**

Adapted from MIT Biological Engineering Communication Lab: <https://bit.ly/3tVXhtx>.

**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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