Annotated example of how to write a result section

(shown for a single figure)

Zetsche et al., "Cpf1 is a single RNA-guided endonuclease...", *Cell* 2015

RESULTS

Subheading states conclusions

Cpf1-Containing CRISPR Loci Are Active Bacterial Immune Systems

Cpf1 was first annotated as a CRISPR-associated gene in TIGRFAM (http://www.jcvi.org/cgi-bin/tigrfams/HmmReportPage.cgi?acc=TIGR04330) and has been hypothesized to be the effector of a CRISPR locus that is distinct from the Cas9-containing type II CRISPR-Cas loci that are also present in the genomes of some of the same bacteria, such as multiple strains of *Francisella* and *Prevotella* (Schunder et al., 2013; Vestergaard et al., 2014; Makarova et al., 2015) (Figure 1A). The Cpf1 protein contains a predicted RuvC-like endonuclease domain that is distantly related to the respective nuclease domain of Cas9. However, Cpf1 differs from Cas9 in that it lacks a second, HNH endonuclease domain, which is inserted within the

Section content corresponds to Fig. 1

Experimental rationale + methods description without too much detail

To simplify experimentation, we cloned the *Francisella novicida U112* Cpf1 (FnCpf1) locus (Figure 1A) into low-copy plasmids (pFnCpf1) to allow heterologous reconstitution in *Escherichia coli.* Typically, in currently characterized CRISPR-Cas systems, there are two requirements for DNA interference: (1) the target sequence has to match one of the spacers present in the respective CRISPR array, and (2) the target sequence complementary to the spacer (hereinafter protospacer) has to be flanked by the appropriate protospacer adjacent motif (PAM). Given the completely uncharacterized functionality of the FnCpf1 CRISPR locus, we adapted a previously described plasmid depletion assay (Jiang et al., 2013) to ascertain the activity of Cpf1 and identify the requirement for a PAM sequence and its respective location relative to the protospacer (5′ or 3′) (Figure 1B). We constructed two libraries of plasmids carrying a protospacer matching the first spacer in the FnCpf1 CRISPR array with the 5′ or 3′ 7 bp sequences randomized. Each plasmid library was transformed into *E. coli* that heterologously expressed the FnCpf1 locus or into a control *E. coli* strain carrying the empty vector. Using this assay, we determined the PAM sequence and location by identifying nucleotide motifs that are preferentially depleted in cells heterologously expressing the FnCpf1 locus. 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 (Figures 1C, 1D and S1). The 5′ location of the PAM is also observed in type I CRISPR systems, but not in type II systems, where Cas9 employs PAM sequences that are located on the 3′ end of the protospacer (Mojica et al., 2009; Garneau et al., 2010). Beyond the identification of the PAM, the results of the depletion assay clearly indicate that heterologously expressed Cpf1 loci are capable of efficient interference with plasmid DNA.

Quick description of how assay was interpreted

Statement of findings

Conclusion

To further characterize the PAM requirements, we analyzed plasmid interference activity by transforming *cpf1*-locus-expressing cells with plasmids carrying protospacer 1 flanked by 5′-TTN PAMs. We found that all 5′-TTN PAMs were efficiently targeted (Figure 1E). In addition, 5′-CTA, but not 5′-TCA, was also efficiently targeted (Figure 1E), suggesting that the middle T is more critical for PAM recognition than the first T and that, in agreement with the sequence motifs depleted in the PAM discovery assay (Figure S1D), the PAM might be more relaxed than 5′-TTN.

Transition + rational + methods

Findings

Conclusion

Adapted from MIT Biological Engineering Communication Lab.

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