Cpf1-Containing CRISPR Loci Are Active Bacterial Immune Systems

Cpf1 was first annotated as a CRISPR-associated gene in TIGRFAM 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 [...].

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. [...] 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′). [...] 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. [...] 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.

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),

Conclusion (II).
… 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.

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