

summary of results

In this work, we characterize Cpf1-containing class 2 CRISPR systems, classified as type V, and show that its effector protein, Cpf1, is a single RNA-guided endonuclease. Cpf1 substantially differs from Cas9—to date, the only other experimentally characterized class 2 effector—in terms of structure and function and might provide important advantages for genome-editing applications. Specifically, Cpf1 contains a single identified nuclease domain, in contrast to the two nuclease domains present in Cas9. The results presented here show that, in FxCpf1, inactivation of RuvC-like domain abolishes cleavage of both DNA strands. Conversely, FnCpf1 forms a homodimer (Figure S2B), with the RuvC-like domains of each of the two subunits cleaving one DNA strand. However, we cannot rule out that FnCpf1 contains a second yet-to-be-identified nuclease domain. Structural characterization of Cpf1 RNA-DNA complexes will allow testing of these hypotheses and elucidation of the cleavage mechanisms.

limitation of study

Perhaps the most notable feature of Cpf1 is that it is a single crRNA-guided endonuclease. Unlike Cas9, which requires tracrRNA to process crRNA arrays and both crRNA and tracrRNA to mediate interference (Datsheva et al., 2015), Cpf1 processes crRNA arrays independent of tracrRNA, and Cpf1-crRNA complexes alone cleave target DNA molecules, without the requirement for any additional RNA species. This feature could simplify the design and delivery of genome-editing tools. For example, the shorter (~42 nt) crRNA employed by Cpf1 has practical advantages over the long (~100 nt) guide RNA in Cas9-based systems because shorter RNA oligos are significantly easier and cheaper to synthesize. In addition, these findings raise more fundamental questions regarding the guide processing mechanism of the type V CRISPR-Cas systems. In the case of type II, processing of the pre-crRNA is catalyzed by the bacterial RNase III, which recognizes the long duplex formed by the tracrRNA and the complementary portion of the direct repeat (Datsheva et al., 2015). Such long duplexes

engineering implications

scientific implications

are not present in the pre-crRNA of type V systems, making it unlikely that RNase III is responsible for processing. Further experiments aimed at elucidating the processing mechanism of type V systems will shed light on the functional diversity of different CRISPR-Cas systems.

Cpf1 generates a staggered cut with a 5' overhang, in contrast to the blunt ends generated by Cas9 (Carroll et al., 2013; Anz et al., 2012; Gersbach et al., 2012). This structure of the cleavage product could be particularly advantageous for facilitating non-

homologous end joining (NHEJ)-based gene insertion into the mammalian genome (Morales et al., 2013). Being able to program the exact sequence of a sticky end would allow researchers to design the DNA insert so that it integrates into the genome in the proper orientation. Specifically, in non-dividing cells, in which genome editing via homologous-directed repair (HDR) mechanisms is especially challenging (Chen et al., 2011), Cpf1 could provide an effective way to precisely introduce DNA into the genome via non-HDR mechanisms.

more engineering implications

Another potentially useful feature of Cpf1 that might aid the introduction of new DNA sequences is that Cpf1 cleaves target DNA at the distal end of the protospacer, far away from the seed region. Therefore, Cpf1-induced indels will be located far from the target site, which is thus preserved for subsequent rounds of Cpf1 cleavage. With Cas9, any indel resulting from the dominant NHEJ repair pathway will disrupt the target site, effectively eliminating the possibility of inserting new DNA at that site in that particular cell. In the case of Cpf1, it appears possible that, if the first round of targeting results in an indel, a subsequent round of targeting could yet be repaired via HDR. Future exploration of these and other strategies using Cpf1 and other class 2 effectors is expected to bring solutions for some of the biggest challenges facing genome editing.

The T-rich PAMs of the Cpf1-family also allow for applications in genome editing in organisms with particularly AT-rich genomes, such as *Plasmodium falciparum* (Liu et al., 2013) or areas of interest with AT enrichment, such as scaffold/matrix attachment regions. To date, all characterized mammalian genome-editing proteins require the presence of at least one G (Frost et al., 2014; Jiang et al., 2013), so the T- and TC-dependent PAMs of Cpf1-family proteins expand the targeting range of RNA-guided genome editing nucleases.

The natural diversity of CRISPR systems provides a wealth of opportunities for understanding the origin and evolution of prokaryotic adaptive immunity, as well as for harnessing potentially transformative biotechnological tools. There is little doubt that, beyond the already classified and characterized diversity of the CRISPR-Cas types, there are additional systems with distinctive characteristics that await exploration and could further enhance genome editing and other areas of biotechnology as well as shed further light on the evolution of these defense systems.

summary of implications

forward-looking statement

Leading Discussion Of A Scientific Journal Article

Hussin A.Rothana



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