

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 FnCpf1, 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 (Carmou et al., 2013; Anz et al., 2012; Gershen 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

Yan Bai



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Critical Thinking Within the Library Program John Spencer, Christopher Millson-Martula, 2014-10-20 While academic librarians frequently discuss critical thinking and its relationship to information literacy the literature does not contain an abundance of sources on the topic Therefore this work provides a current and timely perspective on the possible roles of critical thinking within the library program The work contains a variety of approaches likely to benefit the practicing librarian It begins with a review of the literature followed by theoretical approaches involving constructivism and the Socratic method Readers will find pieces on the integration of critical thinking into the first year experience and course specific case studies as well as a selection on a campus wide critical thinking project In each of the pieces librarians are exploring new ways to meet their instructional goals including the goal of teaching critical thinking skills to students across the curriculum This book was originally published as a special issue of *College Undergraduate Libraries* *The London, Edinburgh and Dublin Philosophical Magazine and Journal of Science*, 1921 *Leading & Managing Occupational Therapy Services* Brend Braveman, 2016-02-19 Put theory and research into practice for real world success Here's your introduction to the use of theory research and evidence in guiding your practice as an occupational therapy manager From leadership and supervision to policies program development and continuous quality improvement you'll find complete coverage of the full range of issues and functions managers encounter in the real worlds in which they practice Whatever your role the practical knowledge and the guidance you'll find here will help you become a more effective OT colleague and manager *British Journal of Dental Science*, 1893 **Articles and Discussions on the Labor Question Including the Controversy with Mr. Lyman J. Gage on the Ethics of the Board of Trade** Matthew Mark Trumbull, 1895 **Journal of the Society of Chemical Industry** Society of Chemical Industry (Great Britain), 1902 Includes list of members 1882 1902 proceedings of the annual meetings and various supplements **London, Edinburgh and Dublin Philosophical Magazine and Journal of Science**, 1921 **Handbook of Teaching and Learning Social Research Methods** Melanie Nind, 2023-09-06 This comprehensive Handbook illustrates the wide range of approaches to teaching and learning social research methods in the classroom online in the field and in informal contexts Bringing together contributors from varied disciplines and nations it represents a landmark in the development of pedagogical culture for social research methods **The Journal of Industrial and Engineering Chemistry**, 1913 *The Journal of the Chemical, Metallurgical and Mining Society of South Africa* Chemical, Metallurgical, and Mining Society of South Africa, 1909 **Teaching, Learning, Literacy in Our High-Risk High-Tech World** James Paul Gee, 2017-04-14 This is a profound look at learning language and literacy It is also about brains and bodies And it is about talk texts media and society These topics though usually studied in different narrow academic silos are all part of one highly interactive process human development Gee argues that children will need to be resilient imaginative hopeful and deliberate learners to survive the deeply complex and unpredictable world in which they

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Transactions of the Section on Preventive and Industrial Medicine and Public Health of the American Medical Association American Medical Association. Section on Preventive and Industrial Medicine and Public Health,1924

Becoming a Consumer Psychologist Ashwani Monga,Rajesh Bagchi,2019-11-13 Written by two scholars at the forefront of conducting research on the psychology of consumers and mentoring those new to the field Becoming a Consumer Psychologist provides a guide to what it takes to become a consumer psychologist and achieve success in this area Monga and Bagchi lay out the varied experiences that lead one to be a consumer psychologist in academia marketing or public policy The book discusses the academic route in detail guiding you on how to apply to schools including for a Ph D what courses to take and what to expect during your educational experience and after It also discusses other routes that lead to diverse non academic career paths in which practitioners apply their knowledge about consumer psychology The authors guidance is backed by their own experiences as consumer psychology researchers mentors and journal Associate Editors and the insights that the authors have gathered exclusively for this book from 23 other leading academics and practitioners This book is essential reading for anyone looking to start their career in consumer psychology and for mentors and advisors who are guiding students about career choices **The Illinois Medical Journal** ,1923 **British Medical Journal** ,1925

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