MINIATURE CRISPR-CAS SYSTEM CHARACTERIZATION

Greta Bigelyte[1], Tautvydas Karvelis[1], Joshua K. Young[2], Zhenglin Hou[2], Rimante Zedaveinyte[1], Karolina Budre[1], Sushmitha Paulraj[2], Vesna Djukanovic[2], Stephen Gasior[2], Arunas Silanskas[1], Česlovas Venclovas[1], Virginijus Siksnys[1]

[1] Institute of Biotechnology, Vilnius University, Vilnius, LT-10257, Lithuania
[2] Corteva Agriscience™ Agriculture Division of DowDuPont™, Johnston, IA 50131, USA
greta.bigelyte@bti.vu.lt

In recent years, CRISPR-associated (Cas) nucleases have revolutionized the genome editing field. Being guided by an RNA to cleave double-stranded (ds) DNA targets near a short sequence termed a protospacer adjacent motif (PAM), Cas9 and Cas12 offer unprecedented flexibility, however, more compact versions would simplify delivery and extend application. Here, we present a collection of 10 exceptionally compact (422-603 amino acids) CRISPR-Cas nucleases that recognize and cleave dsDNA in PAM dependent manner. Categorized as class 2 type V-F they come from the Cas14 family [1] and distantly related type V-U3 Cas proteins found in bacteria. Using biochemical methods, we demonstrate that a 5' T- or C-rich PAM sequence triggers double stranded (ds) DNA target cleavage [2]. Based on this discovery, we evaluated whether they can protect against invading dsDNA in *E. coli* and find that some but not all can. Altogether, our findings show that miniature Cas nucleases are functional CRISPR-Cas defense systems and have the potential to be harnessed as programmable nucleases for genome editing.

[1] Harrington LB, Burstein D, Chen JS, Paez-Espino D, Ma E, Witte IP, et al. Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. Science. 2018 Nov 16;362(6416):839-842.

[2] Karvelis T, Bigelyte B, Young JK, Hou Z, Zedaveinyte R, Pociute K, Silanskas A, Venclovas Č, Siksnys V. PAM recognition by miniature CRISPR-Cas14 triggers programmable double-stranded DNA cleavage. BioRxiv 654897 [Preprint]. 2019 May 30 doi: https://doi.org/10.1101/654897.