

論文の内容の要旨

Structure and Engineering of Cas9 from *Francisella novicida*

(*Francisella novicida* 由来 Cas9 の構造解析および構造に基づく機能改変)

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The CRISPR-associated protein Cas9 cleaves double stranded DNA complementary to the guide RNA and has been harnessed for programmable genome editing. Cas9-targeting DNA sequences are restricted by the requirement of a protospacer adjacent motif (PAM) just downstream of the target sequence. The Cas9 orthologs from different microbes have highly divergent lengths and sequences and recognize diverse PAM sequences. This study presents the crystal structure of *Francisella novicida* Cas9 in complex with guide RNA and its PAM-containing target DNA. The structure revealed striking conserved and divergent structural features in the orthologous CRISPR-Cas systems. The author found that FnCas9 recognizes the 5'-NGR-3' PAM and used the structural information to create the FnCas9 variant that recognizes more relaxed 5'-YG-3' PAM, thereby expanding the targetable sequence of the CRISPR-Cas9 toolbox.