Center for Experimental Medicine and Systems Biology

Division of Genome Engineering
ゲノム編集研究分野

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)　真　下　知　士
Associate Professor	Kazuto Yoshimi, Ph.D.	准教授	博士(医科学)　吉　見　一　人

CRISPR-associated (Cas) nucleases are widely used in life sciences and medicine. To overcome technical and patent-related limitations of CRISPR–Cas9, we have developed a novel genome editing platform based on CRISPR–Cas3. We investigate the molecular mechanisms of Cas3-mediated genome editing, including crRNA maturation and Cascade complex formation, to improve editing efficiency and accuracy in human cells and rodent models. We also expand its translational applications in gene therapy and viral diagnostics, establishing CRISPR–Cas3 as a versatile genome engineering tool.

Enhanced CRISPR-Cas3-mediated genome editing using circularized crRNAs

Kouya Mikamo[1], Kazuto Yoshimi[1,2*], Naoko Abe[3], Kohei Takeshita[4], Hiroshi Abe[3], Tomoji Mashimo[1,2*]

[1]Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo

[2]Division of Genome Engineering, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo

[3]Department of Chemistry, Graduate School of Science, Nagoya University

[4]Life Science Research Infrastructure Group, Advanced Photon Technology Division, RIKEN SPring-8 Center

Type I-E CRISPR-Cas3 represents a genome editing technology in which large deletions averaging several kilobases are introduced in target regions. However, its genome editing efficiency varies considerably across targets and cell types, making it difficult to achieve consistent results. Here, we investigated the efficacy and stability of circularized CRISPR RNAs (ccrRNAs) to enhance CRISPR-Cas3-mediated genome editing in human cells. Using *in vitro* single-strand DNA cleavage assays, we demonstrated that ccrRNA induces Cascade complex formation. Significant genome editing activity targeting the *EMX1* and *B2M* genes was observed in cellular assays using K562 cells. Long-read sequencing identified large-scale deletion mutations at the target loci and no off-target effects using ccrRNA. Furthermore, ccrRNAs exhibited extended intracellular stability compared with that for linear crRNAs, resulting in an enhanced editing efficiency. These results demonstrate that ccrRNAs enable accurate, stable, and highly efficient genome editing and support the broader application of the CRISPR-Cas3 system.

CRISPR-based diagnostics for high-sensitivity Mpox detection

Rika Hirano1, Kazuto Yoshimi1,2, Koji Asano1, Kohei Takeshita3, Ken J. Ishii4, Kei Sato5, Tomoji Mashimo1,2

1 Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo

2 Division of Genome Engineering, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo
3 Life Science Research Infrastructure Group, Advanced Photon Technology Division, RIKEN Spring-8 Center
4 Division of Vaccine Science, Department of Microbiology and Immunology, Institute of Medical Science, The University of Tokyo
5 Division of Systems Virology, Department of Microbiology and Immunology, Institute of Medical Science, The University of Tokyo

Monkeypox (Mpox) has emerged as a critical public health challenge, creating an urgent need for rapid, reliable, field-deployable diagnostic tools for outbreak scenarios. Here, we present Kairo-CONAN, a novel CRISPR-Cas3-based point-of-care diagnostic platform for Mpox, engineered for sustainability and portability. This system uses a disposable hand warmer as a stable heat source and incorporates freeze-dried reagents for ambient temperature stability, enabling device-free, sensitive detection by lateral flow assay strips. Utilizing the unique DNA-targeting and cleavage properties of CRISPR-Cas3, we optimized probe DNA configurations for high specificity and designed clade-specific target CRISPR RNAs (crRNAs). Kairo-CONAN has demonstrated rapid, high-sensitivity, specific detection of Mpox virus (MPXV) DNA across multiple clades, including Clade Ia (Congo), Clade Ib (synthetic DNA), and Clade IIb (Tokyo). Kairo-CONAN addresses logistical and environmental challenges to offer a sustainable, cost-effective, field-adapted solution for infectious disease diagnostics, aligning with the 100 Days Mission framework to enhance global outbreak response efforts.

Mechanisms of Cascade complex formation through 5' end-repeat maturation of crRNA in Type I CRISPR-Cas systems

Kazuto Yoshimi, Kohei Takeshita, Koji Asano, Tetsutaro Kimihira, Kouya Mikamo, Rika Hirano, Tomoji Mashimo
[1]Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo
[2]Division of Genome Engineering, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo
[3] Life Science Research Infrastructure Group, Advanced Photon Technology Division, RIKEN SPring-8 Center

Genome editing tools based on class 1 CRISPR, which have different DNA cleavage mechanisms and deletion patterns from class 2 CRISPR-Cas systems such as CRISPR-Cas9, have recently been developed and are contributing to the expansion of genome editing technologies. Type I CRISPR in the class 1 CRISPR system recognizes target DNA sequences by the Cascade complex, which are generated by the interaction of crRNA precursors and multiple Cas proteins. However, the precise mechanisms underlying the formation of the Cascade complex remain unclear, which limits the potential applications of these systems. This study aims to elucidate the mechanism of Cascade formation in type I CRISPR-Cas by analyzing the crRNA processing steps involved in complex formation by the modifications of crRNA and Cas factors.

We first investigated the critical sequence requirements of crRNA precursor with the Type I-E CRISPR derived from *Escherichia coli*. In vitro cleavage assays revealed that the maturation of the 5' repeat sequence of the crRNA precursor is essential for the formation of a functional Cascade complex. Importantly, this mechanism was further validated across multiple CRISPR subtypes, including Type I-C, Type I-D, and even the Type I-F3 Cas transposon systems, suggesting a universally conserved molecular mechanism. In contrast, modified or truncated crRNAs at the 3' repeat sequence maintained its in vitro DNA cleavage activity and robust genome editing activity in human cells, suggesting that the 3' end is not required for maturation, as modified or deleted non-natural crRNAs at the 3' end is non-essential for crRNA maturation. Furthermore, we also revealed that prior reactions of Cas5, Cas6, and Cas7 lead to appropriate processing and Cascade formation.

This study uncovers a novel molecular mechanism of crRNA maturation and Cascade complex formation in Type I CRISPR systems. Our findings significantly advance the understanding of CRISPR-Cas adaptive immune mechanisms and provide critical insights into the strategic modification of crRNA and the continued development of advanced gene editing technologies including base editing, prime editing and epigenome editing tools with type I CRISPR.

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