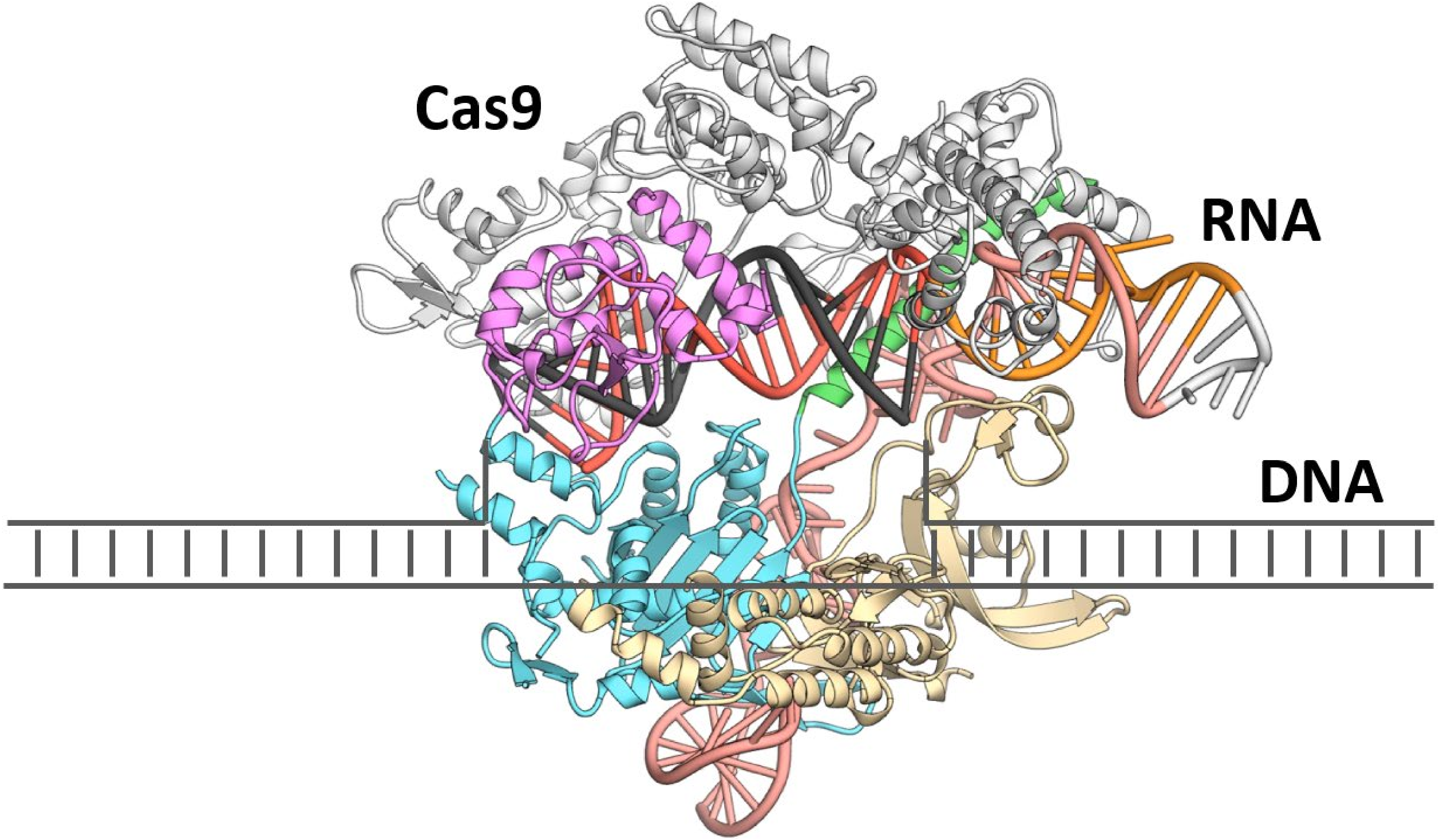
基礎・横断研究戦略
作業部会（第3回）
令和8年3月24日
資料3-4

AMED/BINDS について

東京大学先端科学技術研究センター
教授 西増 弘志

CRISPR-Cas9の立体構造

Cas9がガイドRNAと協働して標的DNAを切断するメカニズムを解明



Nishimasu et al. Cell 2014

2020年8月

東大先端研において独立

教授
准教授
助教
博士研究員
秘書

博士3年　1名 (DC2)
博士2年　1名 (DC2)
博士1年　3名 (1名 DC1)
修士2年　4名 (1名 DC1)
修士1年　4名 (2名 博士進学)
学部4年　4名

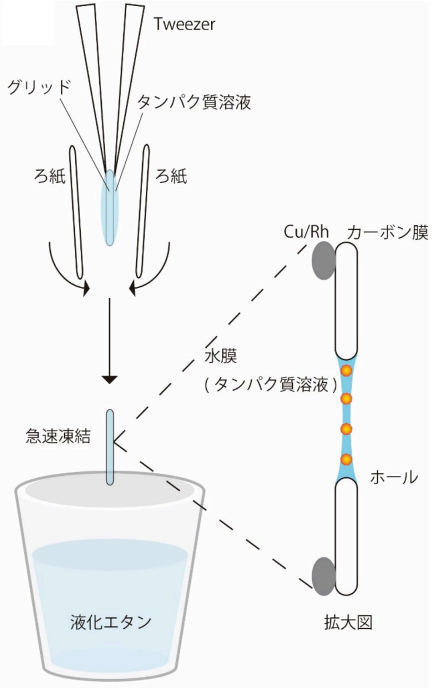
A2-1 タンパク質クライオ電子顕微鏡構造解析支援

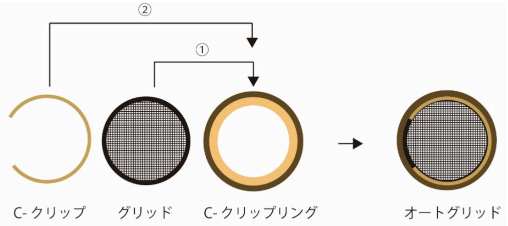
ユニット名

構造解析ユニット

支援担当者

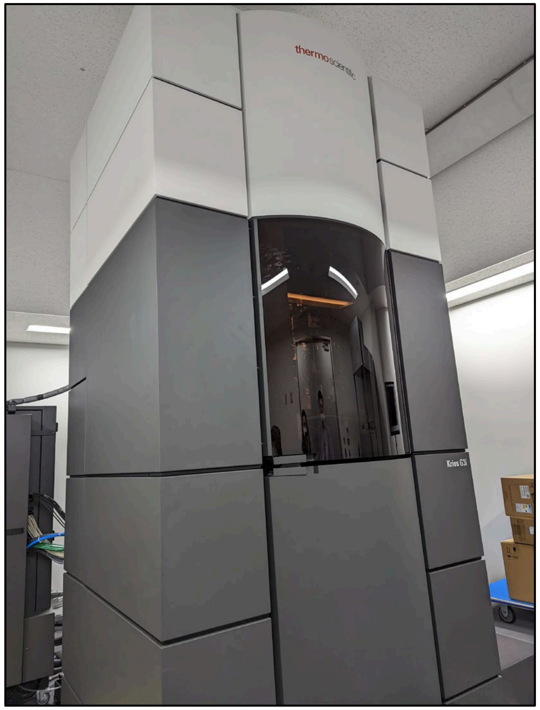
所属		① 東京大学 大学院医学系研究科 ② 東京大学 大学院医学系研究科 ③ 東京大学 大学院医学系研究科
氏名		① 吉川 雅英 ② 齊藤 知恵子 ③ 浜野 文三江
AMED 事業	課題名	クライオ電子顕微鏡による分子・細胞構造解析の支援と高度化
	代表機関	東京大学
	代表者	吉川 雅英





試料作製

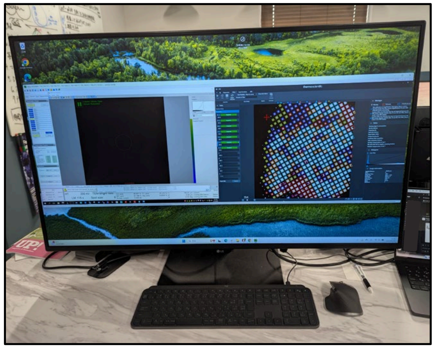
西増研

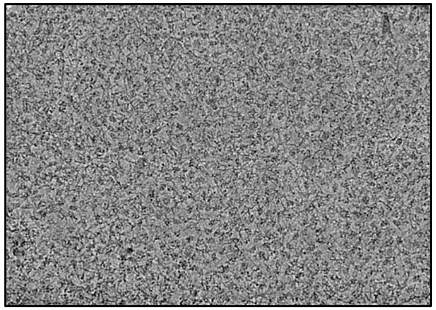


クライオ電顕

電顕観察

医学部研究棟





電顕画像

測定/解析

西増研

AMED/BINDSの支援による成果

1. Structural visualization of the molecular evolution of CRISPR-Cas9.
   Nagahata et al., Nat Struct Mol Biol, 2026

2. Structural mechanism of the Retron-Eco7 anti-phage defense system.
   Ishikawa et al., Nat Commun, 2025

3. Structural insights into RNA-guided RNA editing by the Cas13b-ADAR2 complex.
   Ishikawa et al., Nat Struct Mol Biol, 2025

4. Structural mechanism of bridge RNA-guided recombination.
   Hiraizumi et al., Nature, 2024

5. RNA-triggered protein cleavage and cell growth arrest by the type III-E CRISPR nuclease-protease.
   Kato et al., Science, 2022

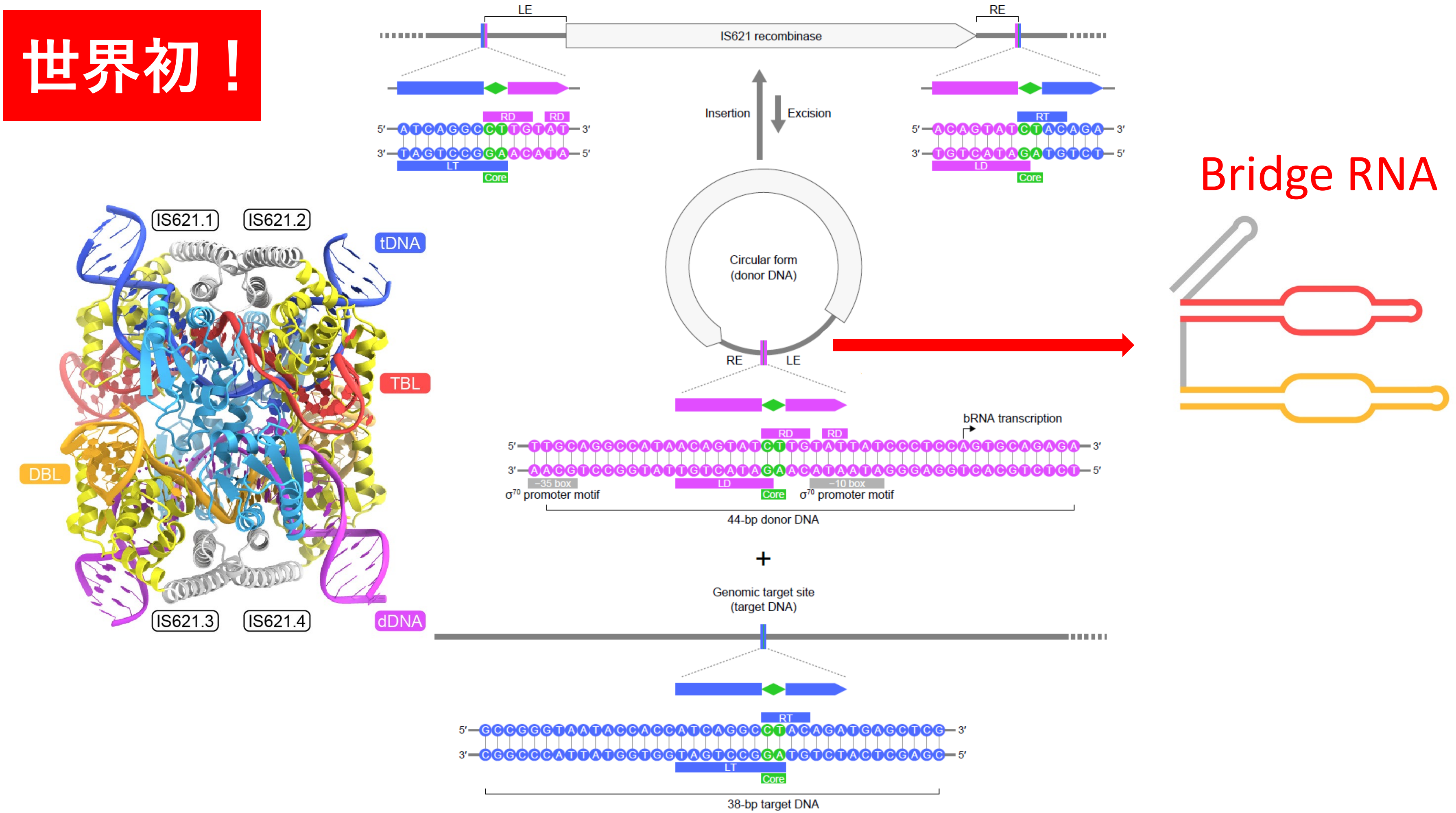
6. Structure and engineering of the type III-E CRISPR-Cas7-11 effector complex.
   Kato et al., Nat Commun, 2022

7. Structure and engineering of the type III-E CRISPR-Cas7-11 effector complex.
   Kato et al., Cell, 2023

非常識にもほどがある！ブリッジRNAが橋渡しするDNA組換えメカニズム

大腸菌の「動く遺伝子」の転移に関与するIS621リコンビナーゼは、
ブリッジRNAと協働して、ドナーDNAのターゲットDNAへの挿入を触媒する

世界初！



Article

Bridge RNAs direct programmable recombination of target and donor DNA

https://doi.org/10.1038/s41586-024-07552-4

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Accepted: 9 May 2024

Matthew G. Durrant[1,2,11], Nicholas T. Perry[1,2,3,11], James J. Pai[1], Aditya R. Jangid[1,2], Januka S. Athukoralage[1], Masahiro Hiraizumi[4], John P. McSpedon[1], April Pawluk[1], Hiroshi Nishimasu[4,5,6,7,8], Silvana Konermann[1,9] & Patrick D. Hsu[1,2,10] ✉

Genomic rearrangements, encompassing mutational changes in the genome such as insertions, deletions or inversions, are essential for genetic diversity. These rearrangements are typically orchestrated by enzymes that are involved in fundamental DNA repair processes, such as homologous recombination, or in the transposition of foreign genetic material by viruses and mobile genetic elements[1,2]. Here we report that IS110 insertion sequences, a family of minimal and autonomous mobile genetic elements, express a structured non-coding RNA that binds specifically to their encoded recombinase. This bridge RNA contains two internal loops encoding nucleotide stretches that base-pair with the target DNA and the donor DNA, which is the IS110 element itself. We demonstrate that the target-binding and donor-binding loops can be independently reprogrammed to direct sequence-specific recombination between two DNA molecules. This modularity enables the insertion of DNA into genomic target sites, as well as programmable DNA excision and inversion. The IS110 bridge recombination system expands the diversity of nucleic-acid-guided systems beyond CRISPR and RNA interference, offering a unified mechanism for the three fundamental DNA rearrangements—insertion, excision and inversion—that are required for genome design.

Evolution has dedicated a vast number of enzymes to the task of rearranging and diversifying the genome. This process enables the emergence and functional specialization of new genes, the development of immunity[3] and the opportunistic spread of viruses and mobile genetic elements (MGEs)[1,2]. MGEs are abundant throughout all domains of life and often mobilize through a transposase, integrase, homing endonuclease or recombinase. These enzymes typically recognize DNA through protein–DNA contacts and can be broadly classified by their target sequence specificity, which ranges from site-specific (for example, Cre and Bxb1 recombinases)[4,5] to semi-random (for example, Tn5 and PiggyBac transposases)[6,7].

Insertion sequence (IS) elements are among the most minimal autonomous MGEs, and are found abundantly across bacteria and archaea. Many characterized IS elements use a self-encoded transposase that recognizes terminal inverted repeats (TIRs) through protein–DNA interactions[8]. IS elements have been categorized into approximately 28 families on the basis of their homology, architecture and transposition mechanisms, but they can be broadly grouped by the conserved catalytic residues of their encoded transposases. These include DDE, DEDD and HUH transposases, and, less frequently, serine or tyrosine transposases[8].

IS110 family elements are cut-and-paste MGEs that scarlessly excise themselves from the genome and generate a circular form as part of their transposition mechanism[9,10]. Given what is known about this mechanism and life cycle, IS110 transposases are more accurately described as recombinases. Although circular intermediates are found in other IS families, IS110 is the only family that uses a DEDD catalytic motif in its recombinase. The N-terminal DEDD domains of IS110 recombinases share homology with RuvC Holliday junction resolvases, suggesting that they have a unique mechanism of action compared with other IS elements. IS110 elements typically lack TIRs and appear to integrate in a sequence-specific manner, often targeting repetitive elements in microbial genomes[11]. Although the mechanism of DNA recognition and recombination for IS110 elements remains unclear, previous studies have suggested that the non-coding ends of the element flanking the recombinase ORF regulate recombinase expression[12,13].

Here we show that the IS110 circular form drives the expression of a non-coding RNA (ncRNA) with two distinct binding loops that separately recognize the IS110 DNA donor and its genomic insertion target site. By bridging the donor and target DNA molecules through direct base-pairing interactions, the bispecific bridge RNA facilitates DNA recombination by the IS110 recombinase. Each binding loop of the bridge RNA can be independently reprogrammed to bind and recombine diverse DNA sequences. We further show that this modularity

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[1]Arc Institute, Palo Alto, CA, USA. [2]Department of Bioengineering, University of California, Berkeley, Berkeley, CA, USA. [3]University of California, Berkeley–University of California, San Francisco Graduate Program in Bioengineering, Berkeley, CA, USA. [4]Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo, Tokyo, Japan. [5]Structural Biology Division, Research Center for Advanced Science and Technology, University of Tokyo, Tokyo, Japan. [6]Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo, Japan. [7]Inamori Research Institute for Science, Kyoto, Japan. [8]Japan Science and Technology Agency, Core Research for Evolutional Science and Technology, Saitama, Japan. [9]Department of Biochemistry, Stanford University School of Medicine, Stanford, CA, USA. [10]Center for Computational Biology, University of California, Berkeley, Berkeley, CA, USA. [11]These authors contributed equally: Matthew G. Durrant, Nicholas T. Perry. ✉e-mail: patrick@arcinstitute.org

Article

Structural mechanism of bridge RNA-guided recombination

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Masahiro Hiraizumi[1], Nicholas T. Perry[2,3,4], Matthew G. Durrant[2], Teppei Soma[1], Naoto Nagahata[1], Sae Okazaki[5], Januka S. Athukoralage[2], Yukari Isayama[5], James J. Pai[2], April Pawluk[2], Silvana Konermann[2,6], Keitaro Yamashita[5], Patrick D. Hsu[2,3,7] ✉ & Hiroshi Nishimasu[1,5,8] ✉

Insertion sequence (IS) elements are the simplest autonomous transposable elements found in prokaryotic genomes[1]. We recently discovered that IS110 family elements encode a recombinase and a non-coding bridge RNA (bRNA) that confers modular specificity for target DNA and donor DNA through two programmable loops[2]. Here we report the cryo-electron microscopy structures of the IS110 recombinase in complex with its bRNA, target DNA and donor DNA in three different stages of the recombination reaction cycle. The IS110 synaptic complex comprises two recombinase dimers, one of which houses the target-binding loop of the bRNA and binds to target DNA, whereas the other coordinates the bRNA donor-binding loop and donor DNA. We uncovered the formation of a composite RuvC–Tnp active site that spans the two dimers, positioning the catalytic serine residues adjacent to the recombination sites in both target and donor DNA. A comparison of the three structures revealed that (1) the top strands of target and donor DNA are cleaved at the composite active sites to form covalent 5′-phosphoserine intermediates, (2) the cleaved DNA strands are exchanged and religated to create a Holliday junction intermediate, and (3) this intermediate is subsequently resolved by cleavage of the bottom strands. Overall, this study reveals the mechanism by which a bispecific RNA confers target and donor DNA specificity to IS110 recombinases for programmable DNA recombination.

Transposable elements are mobile DNA sequences that can move or copy themselves to new locations within genomes. They are widespread throughout all domains of life and have vital roles in shaping genome function and evolution[3]. Transposons typically encode a transposase gene and terminal inverted repeats at both ends of the elements. Using diverse catalytic mechanisms, these transposases recognize the inverted repeats to catalyse the excision and insertion of the transposable element into new target sites in the genome[4].

Insertion sequence elements are the simplest autonomous transposable elements found in prokaryotic genomes and are classified into approximately 30 families[1]. Typical insertion sequence elements are excised and then randomly inserted into new genomic loci by the actions of their cognate transposases. By contrast, the IS110 family elements are excised as circular double-stranded DNA intermediates and then inserted into specific DNA target sequences through unknown mechanisms[5–10] (Fig. 1a and Extended Data Fig. 1a). Given the conservative transposition cycle of IS110 elements, their encoded transposases can be referred to as recombinases, reflecting a closer functional resemblance to site-specific recombinases such as Bxb1 and Cre[11,12]. IS110 family elements consist of a left end, a recombinase-coding sequence and a right end, flanked by the CT (cytosine–thymine) core dinucleotide sequences (Fig. 1a and Extended Data Fig. 1a), and unlike most other insertion sequence elements, they do not encode long terminal inverted repeat sequences, further obscuring their transposition mechanism. Consistent with these unique features of the IS110 elements, the IS110 recombinases differ from other known enzymes and comprise a RuvC-like domain (Pfam PF01548, hereafter referred to as RuvC for simplicity) with a DEDD motif[7], as well as a Tnp domain (Pfam PF02371) with a conserved serine residue[2] (Fig. 1b and Supplementary Fig. 1). These sequence features and functional observations indicate that IS110 elements use a distinctive mechanism of action that has, until now, remained elusive.

In our accompanying study[2], we established that the *Escherichia coli* IS621 element[7], a member of the IS110 family, reconstitutes a σ^{70}-like promoter at the right end–left end junction in its circular intermediate form to express a bRNA from the left end (Fig. 1a and Extended Data Fig. 1a). This bRNA encodes specificity determinants for both donor DNA (dDNA; the IS621 circular form) and target DNA (tDNA; the genomic insertion site) (Fig. 1c and Extended Data Fig. 1a,b). The bRNA target-binding loop (TBL) contains two guide segments: a left target guide (LTG) and a right target guide (RTG), which base pair with the left side of the bottom strand (left target) and the right side of the top strand (right target) of a double-stranded tDNA, respectively (Fig. 1c, Extended Data Fig. 1a,b and Supplementary Fig. 2a). The analogous

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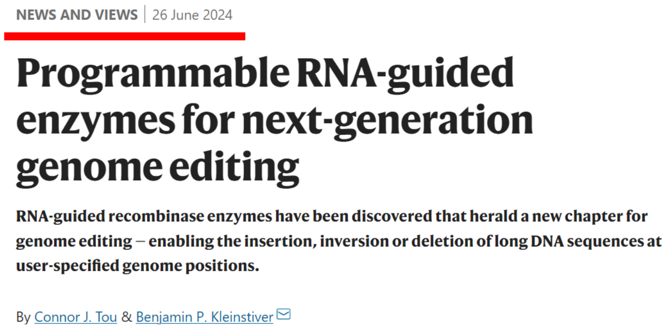
[1]Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, Tokyo, Japan. [2]Arc Institute, Palo Alto, CA, USA. [3]Department of Bioengineering, University of California, Berkeley, Berkeley, CA, USA. [4]San Francisco Graduate Program in Bioengineering, University of California, Berkeley, Berkeley, CA, USA. [5]Structural Biology Division, Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan. [6]Department of Biochemistry, Stanford University School of Medicine, Stanford, CA, USA. [7]Center for Computational Biology, University of California, Berkeley, Berkeley, CA, USA. [8]Inamori Research Institute for Science, Kyoto, Japan. ✉e-mail: patrick@arcinstitute.org; nisimasu@g.ecc.u-tokyo.ac.jp

Durrant, Perry *et al. Nature* 2024

Hiraizumi *et al. Nature* 2024



NEWS AND VIEWS | 26 June 2024

Programmable RNA-guided enzymes for next-generation genome editing

RNA-guided recombinase enzymes have been discovered that herald a new chapter for genome editing – enabling the insertion, inversion or deletion of long DNA sequences at user-specified genome positions.

By Connor J. Tou & Benjamin P. Kleinstiver

TECHNOLOGY FEATURE | 27 June 2024

No CRISPR: oddball ‘jumping gene’ enzyme edits genomes without breaking DNA

A programmable RNA that bridges a genetic donor and a target could herald a safer and more flexible approach to large-scale chromosome changes.

By Heidi Ledford

nature reviews genetics

Research Highlight | Published: 12 July 2024

Genome editing

Programmable DNA rearrangements using bridge RNAs

Henry Ertl

Nature Reviews Genetics **25**, 599 (2024) | Cite this article

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nature chemical biology

Research Highlight | Published: 29 July 2024

Genome editing

RNA-guided recombination

Yiyun Song

Nature Chemical Biology **20**, 941 (2024) | Cite this article

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nature biotechnology

Research Highlight | Published: 14 August 2024

Gene editing

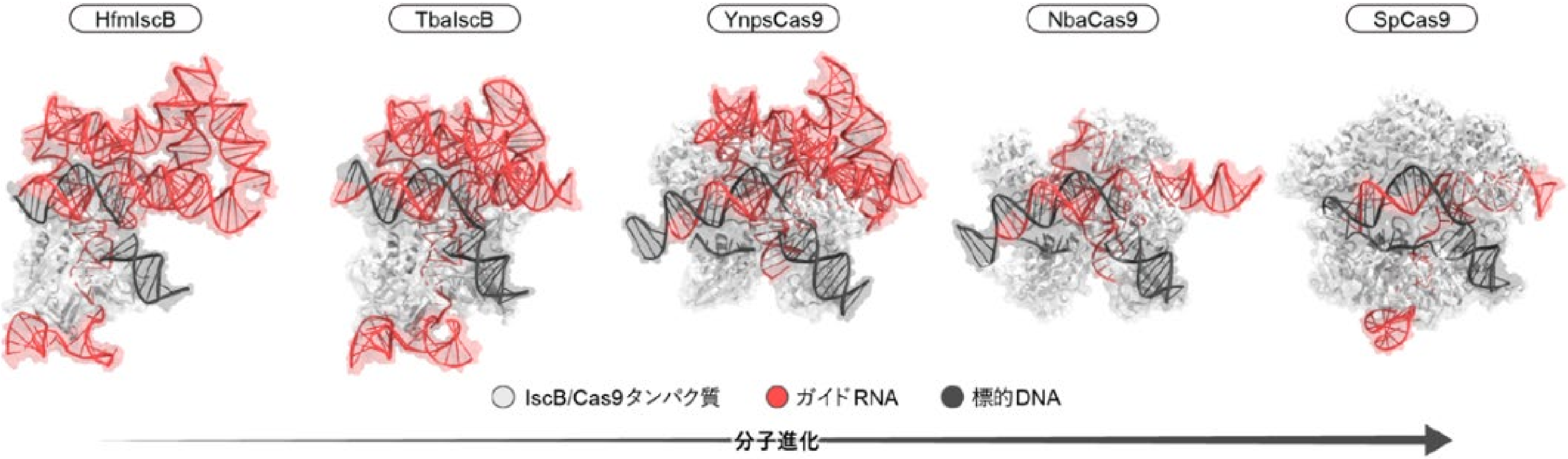
Bridge RNAs direct programmable DNA rearrangements

Iris Marchal

Nature Biotechnology **42**, 1182 (2024) | Cite this article

CRISPR-Cas9の分子進化を可視化！

CRISPR-Cas9の進化中間体と考えられる4種類のIscB・Cas9の
立体構造を決定し、分子進化を明らかにした



Nagahata *et al. Nat Struct Mol Biol* 2026

D3-1 スクリーニング機器・測定装置利用支援

ユニット名

ヒット化合物創出ユニット

支援担当者

所属		① 東京大学 大学院工学系研究科 ② 東京大学 医科学研究所
氏名		① 津本 浩平 ② 長門石 曉
AMED 事業	課題名	創薬モダリティ開発加速及び機能制御分子探索のための物理化学的解析支援
	代表機関	東京大学
	代表者	津本 浩平

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