IMPROVED GENE-EDITING PRECISION TO BOOST JAPAN'S BIOECONOMY

A GENOME-EDITING TECHNIQUE CALLED TYPE I-D (TiD) is based on a lesser-known CRISPR-Cas system and will help engineer the cells that drive the biology-based production of raw materials in Japan.

To kick-start a Japanese bioeconomy in which raw materials are produced using gene-engineered cells, researchers will require access to sophisticated, locally owned CRISPR-Cas-based genome editing tools. A number of teams linked to Japan's Smart Cell Project have been developing these tools since 2016, as part of a national effort to build the research ecosystem needed to commercialize industrially productive cells.

"We want to improve the range and accuracy of available tools, build user-friendly packages, and avoid costly intellectual property issues," says Takahiro Nakamura, a plant molecular biologist from the Faculty of Agriculture at Kyushu University. His group are tasked with working on RNA editing techniques, tool delivery and the intellectual property of the project's genome editing tools.

CRISPR type I-D editing tool

A new tool was recently discovered by a team at Tokushima University's Graduate School of Technology, Industrial and Social Sciences. The tool, dubbed type I-D (TiD), is based on the lesser-known type I CRISPR system.

CRISPR-Cas tools are of two different classes, consisting of six systems and at least 34 subsystems. Globally, many teams are studying the well-characterized systems involved in CRISPR-Cas9, CRISPR-Cpf1 and CRISPR-Cas3, but many CRISPR families are yet to be fully explored as genome editing tools, says Nakamura.

Tokushima University researchers developed their tool by examining Cas effector proteins in the lesser-known type I CRISPR system, says Keishi Osakabe, who leads the group that developed TiD.

CRISPR systems comprise two components: a guide RNA, which is a short RNA fragment essential for complementary binding to the target segment of the genome, and CRISPR-associated (Cas) proteins, which are capable of snipping off targeted DNA fragments near the binding site.

The type I CRISPR systems have some advantages in functionality, explains Osakabe, including longer guide RNA sequences and different mutation profiles.

Typical guide RNAs are about 20 nucleotides long for CRISPR-Cas9 tools, but the TiD system typically uses 35- or 36-base guide RNAs, says Osakabe. Their length may help mitigate off-target effects, one of the main challenges limiting CRISPR technology's practical and commercial potential. The longer guide sequences could potentially be more effectively customized to accurately identify a target sequence, lessening the risk of mutations, deletions, insertions, inversions and translocations.

LONGER GUIDE SEQUENCES COULD POTENTIALLY BE MORE EFFECTIVELY CUSTOMIZED.

The DNA cleavage mechanism is also unique to other common systems, says Osakabe. Cleavage is performed by a specific Cas protein, such as Cas9, Cpf1, and Cas3. However, unlike other CRISPR systems, TiD's Cas10d protein is involved in stabilization, the recognition of the cleavage site, and as the functional nuclease that splits DNA molecules. As a result, TiD can induce both bi-directional long-range deletions and short insertions/deletions. The ability of type I CRISPR to generate such a diverse range of large deletions from a single targeted site could potentially enable long-range chromosome engineering that would allow simple, fast and effective multi-gene function screening studies, says Osakabe.

The potential applications of TiD are being further developed by a multidisciplinary team from Tokushima University, RIKEN, Meiji University, and Kindai University.

Packaged and delivered

The team refer to these engineered cells as 'smart cells' and Nakamura heads one of the Smart Cell Project's other groups currently looking at how to package genome editing tools. He brings experience in developing RNA editing techniques using plant proteins for a successful Japanese start-up, and he points out that, for industry, "it's important to establish user-friendly packages".

To this end, Nakamura aims to establish new base recognition, editing, and delivery techniques for Japanese gene-editing technology. His group has focused on accurate DNA recognition modules, and effective and easily assembled delivery systems for technologies such as TiD.

Nakamura emphasizes that the combination of effective research and real-world savvy will be important to drive interest from industry and develop the right collaborations to harness the current global momentum towards cell-driven production. "Japan's smart cell industry will thrive if it can harness a series of innovative biotechnologies for bioinformatics and metabolomics developed by other Smart Cell Project participants that integrate DNA sequencing, artificial intelligence and machine learning for efficient bio-design."

For this to work, adds Osakabe, new genome editing tools, such as TiD, will be essential to engineer target cells quickly and reliably. This will enable proof-of-concept testing for cell systems identified by the project's other new tools, he says.

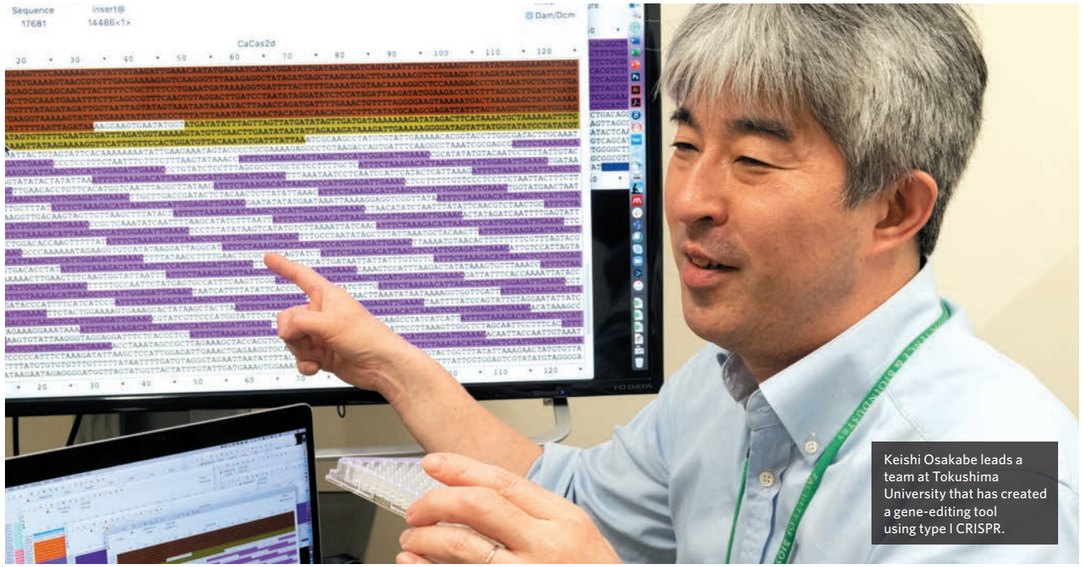
"The convergence of the accomplishments in the Smart Cell Project, including novel genome-editing tools, will be a key feature in its success and the acceleration of the bioeconomy market," he says.

Osakabe's group has already started to apply TiD to plants to increase the production of high-function biomaterials.

This research is part of Japan's Smart Cell Project, which is run by the New Energy and Industrial Technology Development Organization (NEDO). ■

Tokushima University researchers harnessed cutting-edge bioinformatics and genetic engineering.

A researcher working on a new gene-editing tool, type I-D (TiD).



Keishi Osakabe leads a team at Tokushima University that has created a gene-editing tool using type I CRISPR.

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