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Genome editing in plants using small Cas9 and Cpf1

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Cas9 is a RNA-guided endonuclease (RGN) that belongs to CRISPR/Cas system, functioning in an adaptive immune system of bacteria. CRISPR/Cas9 has been intensively developed and applied to genome editing in various organisms. Most widely used Cas9 is from *Streptcoccus pyogenes* (SpCas9) since it robust genome editing activity. SpCas9 gene is 4.1 kb in size and protein recognizes NGG as protospacer adjacent motif (PAM) which is located just next to target sequence. The size and PAM of SpCas9 could restrict the utilization of virus vector and number of target sequence, respectively. To expand the utility of Cas9 protein in plant genome engineering, we tried to adapt two different RGNs to genome editing in plant. One is Cas9 from *Staphylococcus aureus* (SaCas9). The feature of SaCas9 is the smallness in size. SpCas9 consists of 1368 amino acids. In contrast, the number of amino acids of SaCas9 is 1053. Therefore, SaCas9 could be mounts on virus vector. The other is the CRISPR from *Prevotella* and *Francisella* 1 (Cpf1) which is an emerging RGN. Cpf1 has two distinct properties that distinguish it from Cas9. First, Cpf1 utilizes a thymidine (T)-rich PAM, in contrast to the guanidine (G)-rich PAM preferred by Cas9. Thus, Cpf1 can target T-rich regions that Cas9 hardly access. Second, upon cleavage of the target sequence, Cpf1 produces a staggered (sticky) DNA end with a 5′ overhang while Cas9 generates a blunt DNA end. Sticky DNA ends are thought to be applicable to precise genome engineering via a precise end-joining pathway. To evaluate whether these RGNs can be adapted to plant genome editing, we applied SaCas9 and FnCpf1 to targeted mutagenesis in tobacco and rice. Our results demonstrate that SaCas9 and FnCpf1 can be applied successfully to genome engineering in plants.

Biography

Akira Endo is a Post-doctoral Research Fellow at the Plant Genome Engineering Research Unit, National Agriculture and Food Research Organization (NARO). He received his PhD from Tokyo Metropolitan University where he studied about regulatory mechanism of a plant hormone, abscisic acid biosynthesis under drought stress in Arabidopsis using biochemical and immunohistochemical techniques. At NARO, his research has focused on genome editing with CRISPR/Cas9 and CRISPR/Cpf1 systems, and he is currently working on the development of precise genome editing using Cpf1 to create crops with improved agronomical value and increased social acceptance.

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